

REPUBLIC OF TURKEY
YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

THE POTENTIAL USE OF BY-PRODUCTS FROM COLD
PRESSED OIL INDUSTRY AS NATURAL FAT REPLACERS AND
FUNCTIONAL INGREDIENTS IN SALAD DRESSINGS

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DOCTOR OF PHILOSOPHY THESIS

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A thesis submitted by Zeynep Hazal TEKİN ÇAKMAK in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY is approved by the committee on 12.11.2021 in the Department of Food Engineering, Food Engineering Program.

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Zeynep Hazal TEKİN ÇAKMAK

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Dedicated to my family

and my husband

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LIST OF SYMBOLS

A_{control}	Absorbance value of the sample-free solution
A_{sample}	Absorbance value of the sample-containing solution
ω	Angular Velocity
$^{\circ}\text{C}$	Celcius Degree
K	Consistency Index
G_e	Equilibrium Storage Modulus
n	Flow Behavior Index
E_H	Height of Total Emulsion
O_H	Height of Oil Phase
Hz	Hertz
G_0	Initial Storage Modulus in the third time interval
G''	Loss Modulus
μm	Micrometer
mV	Millivolt
d_{32}	Particle size
Pa	Pascal
PdI	Polydisperse index
R^2	Regression Index
γ	Shear Rate
τ	Shear Stress
G'	Storage Modulus
k	Thixotropic rate constant
a_w	Water activity
$\zeta\text{-potential}$	Zeta Potential

LIST OF ABBREVIATIONS

BOB	Black Cumin Oil By-Product
BI	Browning Index
CCD	Central Composite Design
COB	Coconut Oil By-Product
CUPRAC	CUPric Reducing Antioxidant Capacity
EYP	Egg Yolk Powder
ESI	Emulsion Stability Index
FOB	Flaxseed Oil By-Product
GAE	Gallic Acid Equivalents
HF-SD	High-fat salad dressing
LVR	Linear viscoelastic region
LF-SD	Low-fat salad dressing
BOBLF-SD	Low-fat salad dressing enriched with BOB
COBLF-SD	Low-fat salad dressing enriched with COB
FOBLF-SD	Low-fat salad dressing enriched with FOB
POBLF-SD	Low-fat salad dressing enriched with POB
O/W	Oil-In-Water
POB	Pumpkin Seed Oil By-Product
RSM	Response Surface Methodology
3-ITT	Three Interval Thixotropy Test
TPC	Total Phenolic Content
XG	Xanthan Gum

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The potential use of cold pressed oil industry by-products as natural fat replacers and functional ingredients in salad dressings

Zeynep Hazal TEKIN ÇAKMAK

Department of Food Engineering

Doctor of Philosophy Thesis

Supervisor: Assoc. Prof. Dr. Salih KARASU

In this study, the potential use of cold-pressed black cumin seed (BOB), coconut (COB), flaxseed (FOB), and pumpkin seed oil by-products (POB) as functional ingredients and natural oil substitutes in low-fat salad dressing was examined. In the first stage of the study, by-product characterization was carried out. For this purpose, physicochemical properties, bioactive properties, and antimicrobial activities, and in-vitro bioaccessibility of TPC (total phenolic compound) of by-products were investigated. FOB and BOB showed higher TPC and CUPRAC (CUPric reducing antioxidant capacity) values and a higher antimicrobial effect than the other samples. In the second stage, the rheological properties of low-fat salad dressings prepared with 1-5% by-products were compared with low-fat and high-fat salad dressing samples in terms of rheological properties. Salad dressings enriched with by-products exhibited shear-thinning (pseudoplastic), viscoelastic solid behavior, and thixotropic properties similar to that of full-fat salad dressings. In the third stage, 17 different formulations were prepared with 1.0-5.0% POB and 0.5-1.5% FOB. Optimum FOB and POB amounts were determined as 1.49% and 3.04%, respectively by response surface methodology (RSM) and full factorial

trial design (CCD). In the final stage, high-fat salad dressing (HF-SD), low-fat salad dressing (LF-SD), low-fat salad dressing enriched with BOB (BOBLF-SD), with COB (COBLF-SD), with FOB (FOBLF-SD), and with POB (POBLF-SD) were prepared. The emulsion stability (by thermal loop test and phase separation tests), zeta potential and particle size, microstructure, and oxidative stability of the samples were investigated. Low-fat salad dressings prepared with by-products showed higher IP and ΔG^{++} and lower ΔS^{++} values than full-fat and low-fat control samples, indicating that these by-products could improve the oxidative stability of low-fat salad dressings. This study shows that cold-pressed black cumin seed, coconut, flaxseed, and pumpkin seed oil by-products can be used as natural fat substitutes, stabilizers, emulsifiers, and functional ingredients in low-fat salad dressings.

Keywords: Cold pressed, by-products, low-fat salad dressing, rheology, in-vitro bioaccessibility.

Soğuk sıkım yağ endüstrisi atıklarının salata soslarında doğal yağ ikameleri ve fonksiyonel bileşikler olarak kullanım potansiyeli

Zeynep Hazal TEKİN ÇAKMAK

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Bu çalışmada, soğuk presleme ile elde edilen çörekotu tohumu (BOB), hindistancevizi (COB), keten tohumu (FOB) ve kabak çekirdeği yağı yan ürünlerinin (POB) az yağlı salata sosunda fonksiyonel bileşen ve doğal yağ ikameleri olarak kullanım potansiyeli araştırılmıştır. Çalışmanın ilk aşamasında yan ürün karakterizasyonu gerçekleştirilmiştir. Bu amaçla, yan ürünlerin fizikokimyasal özellikleri, biyoaktif özellikleri ve antimikrobiyal aktiviteleri ve TFM (toplam fenolik madde) in vitro biyoerişilebilirliği araştırılmıştır. FOB ve BOB, diğer numunelere göre daha yüksek TFM ve CUPRAC (Bakır(II) iyonu indirgeyici antioksidan kapasite) değerleri ve daha yüksek antimikrobiyal etki göstermiştir. İkinci aşamada, %1-5 oranında yan ürünlerle hazırlanan az yağlı salata sosu örneklerinin reolojik özellikleri (akış davranışı, dinamikler ve 3-ITT), düşük yağlı ve yüksek yağlı salata sosu örnekleri ile kıyaslanmıştır. Yan ürünlerle zenginleştirilmiş salata sosu örnekleri, tam yağlı salata sosuna benzer yüksek kesme incilmesi (psödoplastik), viskoelastik katı davranış ve tiksotropik özellikler sergilemiştir. Üçüncü aşamada %1.0-5.0 POB ve %0.5-1.5 FOB ile 17 farklı formülasyon hazırlanmıştır. Yanıt yüzeyi metodolojisi (RSM) ve tam faktöriyel

deneme tasarımı (CCD) ile optimum FOB ve POB miktarları sırasıyla %1,49 ve %3,04 olarak belirlenmiştir. Son aşamada, yüksek yağlı salata sosu (HF-SD), az yağlı salata sosu (LF-SD), BOB ile (BOBLF-SD), COB ile (COBLF-SD), ile FOB (FOBLF-SD) ve POB ile zenginleştirilmiş az yağlı salata sosu (POBLF-SD) hazırlanmıştır. Örneklerin emülsiyon stabilitesi (termal döngü testi ve faz ayırma testleri ile), zeta potansiyeli ve partikül boyutu, mikroyapı ve oksidatif stabilitesi incelenmiştir. Yan ürünlerle hazırlanan az yağlı salata sosu numuneleri, tam yağlı ve az yağlı kontrol numunelerine göre daha yüksek IP ve ΔG^{++} ve daha düşük ΔS^{++} değerleri göstermiştir, bu da yan ürünlerin az yağlı salata soslarının oksidatif stabilitesini iyileştirebileceğini göstermiştir. Bu çalışma, soğuk presleme ile elde edilen çörekotu tohumu, hindistancevizi, keten tohumu ve kabak çekirdeği yağı yan ürünlerinin az yağlı salata soslarında doğal yağ ikameleri, stabilizatörler, emülgatörler ve fonksiyonel bileşenler olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Soğuk presleme, yan ürünler, düşük yağlı salata sosu, reoloji, in-vitro biyo-erişilebilirliği.

The current consumer tendency towards the least processed food products and the synthetic preservatives so that the nutritional, functional, and sensory properties and quality of cold-pressed oils are better preserved compared to refined oils so that cold-pressed oils may be an alternative for consumers. In cold-pressed oil production, no solvents and no further processing such as excess heating are involved since the cold-pressed is a mechanical pressing that is only followed with filtering. The cold-pressed oil extraction has been reported a suitable method that this method provides oil production rich in bioactive compounds such as polyphenols, essential antioxidants, flavonoids, and sterols, essential fatty acids (particularly α -linolenic acid), carotenoids, tocopherols, sterols, and other hydrocarbons [1-3]. The formation of trans fatty acids and some undesirable components is not a concern since the refining process is not carried out [3]. Again, the cold pressing technique can be environment-friendly and require less energy [4].

In this country, the level of interest in cold-pressed oils has increased considerably in recent years by consumers, and they are called "precious oils" due to their several advantages. However, the use of cold-pressed oils in deep-fat frying, frying, and baking products requiring high-temperature heat treatment results in several reductions in their valuable nutritional and sensory qualities. Even though the cold-pressed oils are notoriously health-conscious consumers in this country, the fact that their consumption is not wide enough to reduce the likelihood that they will benefit from these products in terms of both producers and consumers. After the oil is extracted by cold-pressed, the obtained by-products are dried and rich in nutritional components such as protein and carbohydrates [5]. Therefore, these by-products could also be assessable as fat substitutes in the food industry [6]. In this study, the cold-pressed oil by-products (black cumin, coconut, flaxseed, and pumpkin seed) were obtained from Oneva Foodstuffs and Printing Materials Foreign Trade Ltd Co for use in the food industry as fat mimetics.

Flaxseed has the potential to be a functional food since it contains a high quantity of dietary fiber (two-thirds of which is insoluble and one-third of which is soluble fiber), as well as high-quality protein and an excellent source of unsaturated fatty acids. Flaxseed has a high amount of polysaccharides and proteins, which give it important properties like emulsifying ability, emulsion stabilization, water-holding capacity, surface or interfacial properties, particle suspension, crystallization control, encapsulation, film formation, and thickening and gelling agent [7, 8]. The American National Cancer Institute determined flaxseed as one of the six plant materials that it included among the cancer-preventing foods and predicted to be studied. Flaxseed oil is one of the best sources of α -linolenic acid (ALA), one of the omega 3 (n-3) fatty acids that make up approximately 55% of fatty acids. Interest in flaxseed started after it was realized that the oil obtained by cold pressing contains 50% omega-3 fatty acids [9]. Also, flaxseed oil may be beneficial for the treatment of insulin resistance, Type 2 diabetes, and cardiovascular disease [9]. By-products obtained after the cold-pressed extraction, are products with high nutritional and rheological properties such as oil replacers, emulsifiers, and stabilizers. Flaxseed oil by-products can be preferred in the food industry due to the aforementioned characteristics. Many studies have been conducted on the impacts of flaxseed gum on the rheological properties of an oil-in-water emulsion (O/W) [10, 11]. However, no studies on the use of FOB as a fat replacer in a low-fat salad dressing have been conducted.

Pumpkin seeds may contain 28–38 wt % of oil, which is mainly composed of linoleic, oleic, and palmitic acids [12, 13]. In addition to oil content, considered the number of proteins, crude fiber, minerals, phytosterols, tocopherols, and carotenoids (especially β -carotene and lutein) is found in seeds [14, 15]. Pumpkin seed oil is generally extracted by cold-pressed processing so that it can be categorized as a group of very expensive and good-quality edible oils [16]. Rabrenović, Dimić, Novaković, Tešević and Basić [16] investigated the most significant bioactive compounds of different cold-pressed pumpkin seed oils (*Cucurbita pepo* L.) and they discovered that these oils have high-quality properties, including high levels of sterols (between 718.1 and 897.8 mg/100 g of oil) and especially squalene (between 583.2 and 747 mg/100 g of oil), total

tocopherols (38.03 ± 0.25 - 64.11 ± 0.07 mg/100 g of oil), and monounsaturated fatty acids (between 37.1 and 43.6 g/100 g of total fatty acids). Pumpkin seed oil by-products may be also used in enhancing functional food products thanks to cold-pressed oil production conditions. According to the data obtained by Apostol, Berca, Mosoiu, Badea, Bungau, Oprea and Cioca [17], the partially defatted pumpkin seeds (12.28 g/100g based on d.b.) were confirmed to be a good source of bio-compounds, particularly the total fiber (26.64 g/100g based on d.b.) and formulated functional foods as well as nutraceuticals with known potential prebiotic properties. Pumpkin polysaccharides are water-insoluble and organic solvent-soluble macromolecular substances with essential biological functions and mainly containing galactose, glucose, arabinose, xylose, and glucuronic acid [18]. Wang, Cheng, Liu, Li, Yu, Xu and Yang [19] extracted PSP-1, a new polysaccharide was obtained from pumpkin seeds. This polysaccharide had a molecular weight of 3728 g/mol and was composed of mannose, glucose, and galactose in the following molar ratios: 1.00:4.26:5.78. Dietary fiber, a non-starch polysaccharide, has a variety of functions, including water retention, oil retention, emulsification, and gel formation. Dietary fiber serves to change the textural characteristics of food, avoid syneresis, and stabilize high-fat food and emulsions by incorporating it into the product [20].

Black cumin or black seed (*Nigella sativa* L.) belonging Ranunculaceae family is mostly cultivated in Afyonkarahisar, Burdur, Isparta, and Konya regions in our country. The chemical composition of black cumin seeds is oils (39%), proteins (23%), dietary fibers (16%), starches (15%), crude fibers (5.4%), ashes (4.3%), and moisture (7%) [21]. On the other hand, black cumin seed oils are composed of mostly linoleic acids (50-60%), oleic acids (20-23.4%), palmitic acids (12.5%), acids dihomolinoleic acids (10%), and eicosadienoic acids (3%). Thymoquinone, phytosterols, tocopherols, and essential fatty acids are abundant in this oil [22]. Studies on the pharmacological properties of the black cumin seed oil have been conducted. Black cumin can be utilized as both a nutraceutical and a medicinal seed to treat several diseases such as hypertension, diabetes, inflammation, asthma, cough, influenza, fever, headache, and eczema for decades [23, 24]. The cold-pressed extracted black cumin oil has been also used in the food industry and

pharmaceutical industry. Furthermore, black cumin seed oil has significantly higher oxidative stability than other vegetable oils, due to containing phenolic components, tocopherols, and sterols. Therefore, these oil by-products may be used as a functional agent for low-fat salad dressings.

Coconut possesses many unique physiological roles for human nutrition. Coconut has a wide variety of health benefits due to its fiber and nutritional content. Virgin coconut oil, obtained without using any chemicals and applying high temperatures, is very rich in minerals, vitamins, and antioxidants and is also an excellent nutraceutical. Yalegama, Karunaratne, Sivakanesan and Jayasekara [25] reported that virgin coconut oil residue/by-product contains carbohydrates (39.1%), crude fibre (13.0%), protein (12.6%), fat (9.2%), sugar (13.7%), moisture (4.2%), ash (8.2%). This by-product is within the reported values of potential dietary fiber sources. Therefore, virgin coconut oil by-products can be accepted as a potential dietary fiber source. In the study conducted by Naik, Raghavendra and Raghavarao [26] coconut skimmed milk and insoluble proteins were obtained as by-products after virgin coconut oil production. These by-products were utilized to produce coconut protein powder. Because of its emulsifying characteristics, coconut protein powder may also be used as a food ingredient. Another research found that coconut cake, a by-product of milk and oil extraction, provides a significant quantity of protein. Therefore, Rodsamran and Sothornvit [27] investigated the protein extraction from the coconut by-product. They found glutelin to be the most abundant protein in both coconut by-products (82.31-89.59 %), whereas albumin and globulin had relatively low protein levels.

It is necessary to use the by-products that are produced in oil production in the cold-pressed extraction and have very valuable properties as mentioned above in the food industry of our country and to provide added value to this sector. Therefore, considering the valuable components contained in these by-products, their use in various formulations in the commercial production of food products will reveal significant benefits and advantages. In this study, it is aimed to produce low-fat salad dressings created with new formulations using in formulations to be developed in the production of industrial salad dressing, considering the necessity

of developing new products to ensure the recycling of by-products generated in cold-pressed oil production to the food sector as added value. In this study, it was purposed to obtain cold-pressed flaxseed, black cumin seed, pumpkin seed, and coconut oil by-products and to use cold-pressed by-products in salad dressing production technology with the addition of them in different amounts in formulations.

By-products obtained after the production of pumpkin seeds, black cumin seeds, flaxseed, and coconut oils are materials rich in bioactive substances, especially phenolic substances. These substances have antioxidant, antiradical, and antimicrobial properties due to the bioactive components in their content. In this respect, the products obtained from these by-products have the potential to be used as antioxidant or antimicrobial additives in many foods. Antioxidant substances can be preferred to delay the oxidation of high-fat-containing foods such as mayonnaise and salad dressing. Today, however, the fact that most of these antioxidants are of synthetic origin, the amount of use is limited and the quality of life increases, the consumer preference turns to natural products, making it necessary to seek alternatives. Cold-pressed oil by-products have high bioactive compounds since they are produced without high heat treatment (more than 50 °C) and solvent extraction (e.g. hexane). In addition, the solubility of bioactive compounds in water and their ability to be employed in aqueous extracts will permit these compounds to be utilized in a variety of food compositions. In this study, it is aimed to produce functionally enriched low-fat salad dressing by determining the bioactive components, antimicrobial properties, in-vitro bioaccessibility properties of the by-products of the cold-pressed oil industry.

Recently, consumer preference has concentrated on processed foods such as salads with the increase in vital quality. As a result of this situation, consumption of O/W emulsions which are mayonnaise, salad dressing, and sauces has increased significantly. Salad dressings are a supplementary and snack food group that may be marketed in a wide range of formulations containing spices, oils, acidifying chemicals, and additives [28]. While mayonnaise includes vegetable oil(s) (minimum 65%), salad dressings contain mostly vegetable oil (at least 30 %) USFDA [29]. The salad dressings are prepared without heat treatment and

consumed cold, which is why the cold-pressed oils are suitable for salad dressings. On the other hand, overconsumption of fat or high-calorie meals has been related to several health problems, especially obesity and cardiovascular disease [30]. As a result, lowering fat content is critical for health advantages, even if removing fat produces major changes in the sensory and majority physicochemical characteristics of O/W emulsions that may be unpleasant to consume [31].

Salad dressings are semi-solid emulsified products that are used to improve some properties of salad like, texture, lubricity, flavor, and color [31]. Vegetable oil (at least 30%), stabilizer, emulsifier, spices, acidifying ingredients (vinegar), and additives were used to develop salad dressing type emulsions [31]. In comparison to protein (4cal/g) or carbohydrates (4cal/g), fat provides more energy (9cal/g). As a result of customer awareness, the food industry has started to produce low-calorie food products. However, the food industry faced significant challenges in trying to reduce its fat content. Also, food scientists have studied to develop reduced-fat, low-fat, or fat-free food products with similar pourability, appearance, flavor, texture, rheological and sensory properties of full-fat products [30]. Fat-based fat mimetics can be preferred to prepare reduced-fat salad dressings due to emulsifying properties, desirable mouthfeel, and flavor. Carbohydrate-based ones, on the other hand, provide increasing viscosity, desired mouthfeel, and better texture properties, whereas protein-based ones have been used to improve texture and mouthfeel [31]. Different fat replacers that improve the rheological properties of low-fat food products should also be investigated in order to satisfy mouthfeel perceptions of low-fat O/W emulsions.

The components used in the formulation are directly effective in determining the quality of the salad dressing. Oil, emulsifiers, and stabilizers are components that have a significant influence on the structural qualities of food products [32]. First and foremost, the component ratios should be optimum to obtain a product with the desired level of structural qualities. Oils are one of the most essential compounds that influence the rheological, textural, emulsion stability, oxidative stability, and sensory properties of salad dressings. Increasing the fat content of food products causes an improvement in rheological properties such as viscosity, viscoelastic structure, and consistency, however, the oxidative stability of the food

product may decrease. In addition to the textural and structural properties, the oil type and oil content can directly affect the sensory qualities of the product. The increase in oil content can significantly affect the shelf life of the product. However, oil consumption causes some health problems. Therefore, there has been a trend towards products with reduced oil in recent years.

Emulsifiers play a major role in emulsion stability by reducing the oil-water interfacial tension in oil emulsions dispersed in water, such as salad dressing. Adjusting the amount of emulsifiers correctly is very significant in terms of emulsion quality. The amount of emulsifier should reach a concentration that can form a film around the oil particle. If the emulsifier concentration is not at this level, an interaction begins between the oil particles and a decrease in emulsion stability may be occurred during the storage period. On the other hand, if the amount of emulsifier is used excessively, that is, if the rate of emulsifier that is not adsorbed around the oil particle is too high, another flocculation mechanism (Exhaustion flocculation) comes into play, and the emulsion stability decreases [33]. Stabilizers interact with water in the continuous phase and cause an improvement in the viscosity and other viscoelastic characteristics of the continuous phase. This reduces the mobility of the dispersed oil particles in the continuous phase and improves emulsion stability [31, 34]. In addition, the negative charge of stabilizers at acidic pH values, such as in salad dressings, increases the zeta potential and a stable dispersion structure is formed thanks to the electrostatic repulsion force between the particles [35]. In addition to these benefits, the application of stabilizers in high concentrations results in a significant rise in the viscosity of the continuous phase, as well as impairment in the flow behavior, viscoelastic properties, and recovery qualities of the product. For this, the amount of stabilizer must be within certain limits. Therefore, it is necessary to optimize the amounts of the salad dressing ingredients to produce a salad dressing with a high consumption quality, emulsion stability, and flow properties [31, 36]. In this study, cold-pressed oil by-products may cause gel formation by holding water in salad dressing samples due to the polysaccharide contents. Thus, they can cause an increase in the consistency coefficient by acting as a stabilizer. Also, cold-pressed oil by-products can be emulsifying due to the protein contents. For

this reason, it is thought that our study will contribute to the production of low-fat salad dressing with quality properties similar to high-fat salad dressing, thanks to the emulsifier and stabilizer properties of cold-pressed oil by-products.

Salad dressing rheological analysis has been studied in the literature as a suitable formulation, process conditions, and quality characteristics, sensory qualities, shelf life, and microstructure are significant factors in the selection of salad dressing [37]. There has been a lot of studies performed on the steady-state and dynamic rheological characteristics of salad dressings [28, 38-40]. Only one research has been found in the literature on the 3-ITT rheological properties of the salad dressing formulated with cold-pressed extracted chia seed oil by-products [6]. There has been no research conducted for thixotropic behavior (3-ITT) and other rheological properties of salad dressings formulated with other cold-pressed oil by-products. Also, the emulsion stability of low-fat salad dressing prepared with cold-pressed oil by-products will be firstly determined by a thermal loop test.

The main purpose of this study is to determine the possible use of the cold-pressed oil by-products (BOB, COB, FOB, and POB) in salad dressings as fat replacers and functional agents. For this purpose, the rheological properties of salad dressings formulated with these by-products will be optimized using different formulations based on commercially produced salad dressings. Also, rheological properties, oxidative and emulsion stability, and shelf life levels of the products produced with the optimum formulation will be improved. After optimizing the rheological properties using different formulations, the effects of oil by-products on the emulsion stability, oxidative stability, shelf life, and physicochemical properties of the product were investigated. Thus, the production parameters of the low-fat salad dressings with the highest level of structural qualities and stability were determined.

1.1 Literature review

1.1.1 Cold-pressed oil

Chemical solvent extraction, conventional mechanical extraction, and the most current cold-pressed mechanical technique have all been used to obtain various high-quality edible oils [41]. In the cold-pressed mechanical technique, flakes or even whole seeds are carefully mechanically extracted without having undergone a prior heat treatment so the cold-pressed oils are named as natural and unrefined oils (Guidelines for edible fats and oils of the German Food Codex) [42].

As cold-pressed oils have been produced in a minimum level of a process without any chemical, high heat treatment, and refining process or solvent extraction during production, much less change is observed in the nutritional components compared to refined oils [3]. The cold-pressed oils are nutritional and functional because they contain lipophilic bioactive components such as essential antioxidants and sterols, essential fatty acids (especially α -linolenic acid), phenolic substances, tocopherols, carotenoids, sterols, and other hydrocarbons which vary from raw material to raw material [1, 43]. Since the refining process is not carried out, the formation of trans-fatty acids and some undesirable components is not a concern [3]. The cold-pressing technique can be friendly to the environment and require less energy [4]. In our country, the level of interest in cold-pressed oils has increased considerably in recent years by consumers and they are called "precious oils" thanks to their several advantages.

The cold pressing process does not provide as high extraction efficiency as other extraction techniques with solvent and/or high-temperature treatment. After cold press extraction, a high amount of oil can be obtained, however, the obtained oil has high nutritive components. The storage stability, usefulness, and nutritional properties of cold-pressed oil by-products are all influenced by the residual oil component [44, 45]. The cold-pressed by-products are likewise enhanced in terms of nutritional components such as high protein and carbohydrate, with no trace of solvent [5]. As a result, these by-products must be assessed. There are several research on cold-pressed oil extraction in the literature [5, 16, 41, 42, 46-49].

Emir, Güneşer and Yılmaz [46] investigated that the sensory perceptions, aromatic qualities, and consumer preferences of poppy seed oils obtained by cold pressing from three poppy varieties.

Yang, Zheng, Zhou, Huang, Liu and Wang [47] investigated the total phenolics, tocopherols, phytosterols, lutein, b-carotene, and chlorophyll and the induction period (IP) of cold-pressed oils (203 rapeseed types) from the Yangtze River Valley in China.

Siger, Dwiecki, Borzyszkowski, Turski, Rudzińska and Nogala-Kałucka [48] studied the physicochemical characteristics, bioactive compounds, and fatty acid compositions of the cold-pressed common beech (*Fagus sylvatica* L.) seed oil.

Górnaś, Siger, Juhņeviča, Lācis, Šnē and Segliņa [49] investigated a new cold-pressed oil obtained from Japanese quince seeds (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach, family: Rosaceae). Also, they studied the characterization of Japanese quince seed oils. Its total phenolics content, b-carotene, chlorophyll, antioxidant activity, color parameters, and tocopherols, tocotrienols, and plastochromanol-8 content were compared with nine oils' which are obtained from almonds, flaxseed, hazelnut, peanut, poppy, pumpkin, sesame, sunflower, walnut. In conclusion, Japanese quince seed oil is a distinctive, abundant, and potential source of bio compounds that may be efficiently used in foodstuff.

Ghorbanzadeh and Rezaei [50] investigated the aqueous extraction technique for pomegranate seed oil. RSM was used to investigate the effects of extraction temperature and time, water/solid ratio, and pH on the extraction efficiency of pomegranate seed oil utilizing an aqueous extraction. Finally, the extraction temperature and time, water/solid ratio, and pH all affected the aqueous extraction of pomegranate seed oil.

Cold-pressed pumpkin seed oil (*Cucurbita pepo* L.) is a valuable source of high nutritional value such as bioactive components, which has a positive contribution to human health due to its functional properties. Cold-pressed pumpkin seed oil contain 36–61% linoleic acids (LA; 18:2 n-6, ω -6 fatty acid), 21–47% oleic (18:1 n-9, ω -9 fatty acid), 9–15% palmitic (16:0), and 3–7% stearic (18:0). These oils contain triacylglycerols, vitamins, phytosterols, minerals, carotenoids, and

polyphenols (flavonoids), as well as fatty acids [51]. The regular dietary intake of pumpkin oils provides some health benefits, such as anti-diabetic, anti-inflammation, and lowering of cholesterolemia [52].

Cold-pressed flaxseeds (*Linum usitatissimum* L.) oil contain 51–55% alpha-linolenic acid (ALA; 18:3, ω -3 fatty acid), 16-17% of linoleic (LA; 18:2, ω -6 fatty acid) and 20-21% of oleic acid (18:1, ω -9 fatty acid). The total tocopherol (potential fat-soluble antioxidants) content of flaxseed oil ranged from 39.5 to 50.0 mg/100 g. The flaxseed oil may prevent cardiovascular diseases and blood pressure [53].

Cold-pressed black cumin (*Nigella sativa* L.) oil is high in essential fatty acids as well as bioactive sterols and tocopherols. The main fatty acids in cold-pressed black cumin oil were linoleic acid (LA; 18:2 n-6, ω -6 fatty acid), followed by oleic acid (18:1 n-9, ω -9 fatty acid) and palmitic acid (16:0). Black cumin oil is rich in bioactive thymoquinone, phytosterols, and tocopherols. α -Tocopherol constituted 45% of tocopherols in black cumin oil [23].

Cold-pressed coconut oil (*Cocos nucifera* L.) consisted primarily of 91% saturated fatty acid, myristic acid, lauric acid, and caprylic acid. Cold-pressed coconut oil may have an antioxidant, antiviral and antibacterial activity, reduce total cholesterol, triglycerides [54]. Cold-pressed cardamom, milk thistle, mullein, onion, parsley, roasted pumpkin seed oils were identified by Parry, Hao, Luther, Su, Zhou and Yu [55]. They investigated their total phenolic content (TPC), radical-scavenging capacities against peroxy, DPPH (diphenylpicrylhydrazyl) radicals tocopherol content, carotenoid profile, fatty acid (FA) composition, oxidative stability index (OSI), physical properties, and color were investigated. These seed oils, according to the findings of this study, can be employed in foods as dietary sources of particular fatty acids, phenolic compounds, natural antioxidants, tocopherols, and carotenoids.

1.1.2 Cold-pressed oil by-products

There is less change in bioactive components in cold-pressed oils compared to refined oils since they are not exposed to high heat treatment and solvent treatment during manufacture. As a result, customer preference is focused on

cold-pressed extracted oils [56, 57]. Since they are not subjected to chemical treatment and the food components in their structure are preserved, the use potential of the by-products generated after cold-pressed oil production as a food source is high [45]. Seeds and seeds to be used in cold-pressed oil production are subjected to a heat treatment such as drying before production and the moisture content is reduced depending on the seed variety. In the by-product obtained after oil extraction, proteins and polysaccharides mostly constitute the dry matter [45]. From this point of view, cold-pressed oil by-products can enhance the rheological properties of low-fat food products because of the high protein and carbohydrate content in the cold-pressed oil by-products.

After oil manufacturing, significant amounts of by-products are produced in the cold-pressed oil industry. The recovery of these by-products is critical for lowering financial expenses. It is critical to determine the physicochemical, nutritional, and functional qualities of items to expose their economic and health-improvement potential. According to this study, cold-pressed oil by-products have a valuable potential to be utilized for the enrichment of foodstuffs in many sections of the food industry, and cold-pressed oil by-products can be examined for this purpose.

Karaman, Karasu, Tornuk, Toker, Gecgel, Sagdic, Ozcan and Gül [45] investigated the physicochemical properties, bioactive compounds, fatty acid profile, mineral composition, volatile compounds, and antibacterial activity of different cold-pressed oil by-products (almond (AOB), walnut (WOB), pomegranate (POB), and grape (GOB)). According to the results of this study, by-products include a considerable quantity of protein and bioactive components. It is concluded that GOB and POB can be considered new sources of dietary fiber since they have significant amounts of crude fiber. As a consequence of this research, it was discovered that these by-products had great potential and attributes in many aspects that might be utilized to enhance foodstuffs in various sections of the food industry.

Akcicek and Karasu [6] studied the production of salad dressing utilizing cold-pressed chia seed oil by-products with optimum quality, reduced oil content, with any change in rheological properties. The impact of cold-pressed grape seed oil

by-products on the rheological characteristics of low-fat O/W emulsions was investigated by Karasu, Çetin and Toker [58]. Cold-pressed grape seed oil by-product has a great potential for usage in emulsion products because of its structure-enhancing properties due to its high fiber and polysaccharide content, as well as its oxidative stability-enhancing properties due to its rich phenolic material [59]. This study, it is aimed to produce an emulsion with reduced oil by using a cold-pressed grape seed oil by-product.

1.1.3 Emulsion

An emulsion is a colloid of two or more immiscible liquids, one of which is dispersed as small particles in the other. Effective mixing of these two phases is very important in the production of emulsions with high quality, stability, and sensory properties. The phase that is dispersed as droplets in the other liquid in the emulsion is called the dispersed phase, internal phase, or discontinuous phase, and the phase surrounding the other liquid droplets is called the mobile phase, continuous phase, or outer phase [33].

In food technology, emulsions are most commonly encountered as oil and water emulsions. If oil droplets are scattered throughout the aqueous phase on the emulsion, it is referred to as an O/W emulsion, otherwise, it is mentioned as a W/O emulsion. In the field of food technology, both forms of emulsions are widely used. Examples of oil-in-water emulsions are salad dressings, mayonnaise, other sauces, and ice cream whereas the examples of water-in-oil emulsions are butter, margarine, and cold cream [33]. As mentioned above, in the production of emulsion foods, two different phases are formed: the continuous phase and the dispersed phase. The properties of these phases significantly affect the physicochemical characteristics, stability, and sensory qualities of emulsions. Besides the dispersed phase, the properties of the continuous phase also affect the stability of emulsions. In the continuous phase, there are many components such as salt, starch, gums, acids, and flavor compounds. These components affect the physicochemical properties of emulsions. Stabilizers are effective in maintaining emulsion stability, for example, by increasing the viscosity of the continuous phase. Factors such as the load balance of the medium, polarity, interactions of

the components, their shape and densities, as well as the types and concentrations of the components in the continuous phase, affect the emulsion stability. Therefore, in the production of emulsions, these components should be selected correctly and their ratios should be optimized for a quality product.

In addition to the continuous and dispersed phases, there is the interface region that separates these two phases. The interface is an important factor, especially for emulsions. In the interface region, there are other components such as oil, water molecules, hydrocolloids, emulsifiers, and salts. Although the interface region covers a very small area compared to the total volume of emulsions, the properties of this region critically affect the rheological, microstructural, sensory, and emulsion stability properties of emulsions. Therefore, interface properties are very important in the formation and improvement of emulsions. Since the components used in food processes and formulation directly affect the interface properties, the selection of the components and the optimization of the process parameters are considered in the improvement of the interface properties.

1.1.4 Salad dressing

O/W food emulsions mainly contain ingredients such as oil, emulsifiers, and stabilizers. The physical, sensory, and microbiological quality of emulsion-type products is determined by each component [60]. In salad dressing, while oil, emulsifiers, and stabilizers affect the rheological properties, stability, morphological properties, and particle size of the emulsion; flavors like spices and vinegar affect the sensory quality, and antimicrobial substances affect the product's shelf life. In addition to contributing to these properties, each component separately, their interaction with each other is also very important for the final product quality.

Water is one of the most significant components affecting many properties of salad dressings as it is in all other food ingredients. Stabilizers, emulsifiers, salt, and sugar are all dissolved in water and utilized in salad dressing composition. Water is the continuous phase in O/W emulsions. Salad dressing characteristics which are viscosity, consistency, and emulsion stability are determined with the mobility of the continuous phase. Therefore, the mobility of the aqueous phase is taken

into account in the formulation of such emulsion products and the preparation of the products. This is more important for salad dressings containing relatively less oil. Therefore, the mobility of the aqueous phase is properly considered in the formulation and preparation of certain emulsion products. This is more important for low-fat salad dressings. As it is known, low water activity is a very important feature in terms of shelf life in foods. The a_w value of salad dressings has been reported as 0.93-95 depending on the oil content [31].

Oil is one of the most significant ingredients in salad dressing, impacting its rheological, textural, stability (emulsion and oxidative stability), and sensory properties. The type and amount of oil in the content of salad dressing varies a lot. However, the amount of oil in the formulation is less than mayonnaise and varies between 20-45% [61]. Canola, soy, corn, sunflower, and olive oil are some of the popular types of vegetable oils used in salad dressing preparation. While the amount of oil is increased, the rheological properties which are viscosity, viscoelastic structure, and consistency can be improved, it has the potential to reduce the oxidative stability of the food product. Especially in oil/water emulsions, if the oil ratio falls below 60%, the product stability decreases, and the structure provided by the oils is tried to be compensated by using other substances such as hydrocolloids [34]. In addition to the textural and structural properties, the oil type and fat content can directly affect the sensory properties of the product. However, oils are also important carriers of other bioactive components such as fat-soluble vitamins, coloring agents, and antioxidants. The shelf life of a product can be considerably shortened if the fat content is increased. With the increase in the oil content, the decrease in the amount of the aqueous phase, which we call the mobile phase, will benefit from the decrease in the water activity and thus the shelf life will increase in terms of microbial. Also, with the increase in quality of life in recent years, there is a trend towards products with reduced fat, with the idea that fat consumption will cause some health problems. In recent years, the number of studies on low-fat salad dressing has increased [31].

Many emulsifying agents can be used in the salad dressing formulation. Egg yolk powder, lecithin, mono and diglycerides, vegetable-based proteins (Starch esters, polysorbates), milk-derived proteins (casein, serum proteins), and some

hydrocolloids (gum arabic) are the main emulsifying substances. Emulsions have a thermodynamically unstable structure due to the density difference between oil and water, as well as the surface tension between both molecules. In terms of food technology, it is critical in O/W emulsions that oil particles are distributed in water as tiny droplets and that they form stable dispersions for a long time without interacting with each other. Emulsifiers and stabilizers that lower the surface tension and mobility of the continuous phase are required in the production of thermodynamically stable emulsions. This can be explained that the emulsifiers are located around the oil droplets and acting as a bridge by interacting with water [62]. Although the capacity of emulsifiers to form a good emulsion varies according to their hydrophobic lipophobic balance values (HLB), concentrations, and dispersion capacity, a successful emulsion is closely related to many physicochemical properties of emulsifiers such as surface-active property, holding kinetics, interface tension reduction feature, providing stability, and surface coating [63].

Egg yolk is utilized in many foods due to its functional and organoleptic properties in addition to its emulsifier feature. Egg yolk consists of two basic structures: plasma and granule. Plasma constitutes 78% of the egg yolk. Approximately 85% of plasma is composed of LDL and 15% of livetin protein. The granule consists of 50% protein (phosphite) and 7% oil. The emulsifier properties of plasma and granule sections are different from each other [64]. In salad dressings, egg yolk can be applied directly in liquid egg form or powder form. The method of use also significantly affects the emulsifying capacity of the egg yolk. The ability of egg yolk lipoproteins to form a good emulsion can be explained by a flexible structure, good interaction between oil and water, and reducing surface tension [65].

Products such as salad dressing and mayonnaise are classified as acidic foods. The pH value of the products is below 4.5, often between 3.4-4.0. These values of the pH value of salad dressing can be reached by adding high acidity foods such as lemon juice, especially vinegar, to the formulation or by using citric, tartaric, malic acid solutions. In addition to providing the desired aroma and taste values in salad dressings, acidifying agents also benefit from their microbial growth preventive effects. Low pH values do not fully ensure the safety of salad dressings. From this

point of view, the type of acidifying agent used is also an important factor in providing an antimicrobial effect. For example, although the pH value of lemon juice containing citric acid is low, it does not have an antimicrobial effect. Vinegar containing acetic acid has a certain antimicrobial effect. This is due to acetic acid dissociating in the aqueous phase, which has an antibacterial effect. It has a substantial impact on pathogenic bacteria when the quantity of acetic acid in the aqueous phase surpasses 0.25%.

Sugar and salt are mostly used in products like mayonnaise and salad dressing to capture the product's distinctive flavor. In addition, salt inhibits the development of microorganisms, increases rheological characteristics, and alters the functional properties of proteins. The addition of salt to the formulation improves the stability of the emulsion. The emulsifying characteristics of proteins at the interface are enhanced by the decrease of electrostatic repulsive power between droplets and changes in the hydrophobic interaction of non-polar amino acids. Salt increases the surface-active characteristics of low-density lipoproteins (LDL) in emulsions using egg yolk as an emulsifier [31].

Spices, flavoring agents, and other spices have a crucial impact on product quality and consumer choice, particularly in salad dressing and related emulsion products' sensory characteristics. Salad dressings contain a wide variety of spices and flavors. Most of the flavor compounds in food products are fat-soluble. Flavor sensation may be altered for fat-free salad dressing, as well as for full-fat ones because the distribution of water and fat in a salad dressing typed emulsion affects the balance of each flavor. As the fat content of salad dressing and mayonnaise decreases, the effect of their flavor becomes more intense. Therefore, it can be a major challenge for the food industry to develop such low-fat ingredients to satisfy consumers' demand for oily taste with the required flavor release pattern.

1.1.5 The rheological behavior of salad dressing

Rheology is a straightforward analysis for determining the behavior of solutions, suspensions, and mixes. It is a field that combines continuous mechanics with the ideas obtained by considering the microstructure of the fluid being studied. As is known, rheology measurement is very important in terms of the general process

economy. With rheology measurements, very useful behavioral and predictive information on processing, formulation changes, aging phenomena, and quality for various products can be obtained. Flow behavior is a direct evaluation of workability and an indirect indicator of product uniformity and quality. For example, more pumping power is required to pump a high viscosity fluid than a low viscosity fluid. Therefore, it is necessary to know the rheological behavior of the product to be used when designing pumps and piping systems.

Rheological data in the food industry [66];

- Engineering calculations required for the design of equipment such as pumps, mixers, coating machines, extruders, heat exchangers, and homogenizers or processes related to this equipment in pipelines,
- Determining component functions in product innovations,
- Quality control of intermediate and/or final products,
- In shelf-life testings,
- In the sensory data-based assessment of food structure, and
- In the analysis of rheologically based component equations were needed.

Foods are available in solid, liquid, and semi-solid forms. Liquid foods flow under the effect of gravity and cannot keep their shape [67]. Some foods, such as ice cream, can be solid at one temperature and liquid at another. Fluid foods can have a wide variety of properties, from simple Newtonian behavior to time-dependent non-Newtonian viscoelastic behavior. A foodstuff may exhibit different classes of behavior depending on the conditions. For example, while dilute apple juice shows Newtonian behavior, concentrated apple juice shows a weakening feature with the slip rate [68, 69]. For this reason, rheological properties are classified in Figure 1.1, not foods [68]. As seen in Figure 1.1, viscous behaviors are divided into two as Newtonian and non-Newtonian. Non-Newtonian behaviors are examined in two parts, independent of time and time-dependent. Timeless non-Newtonian behaviors are pseudoplastic, dilatant, Bingham plastic, and Herschel-Bulkley; time-dependent non-Newtonian behaviors are also classified as thixotropic and reopectic.

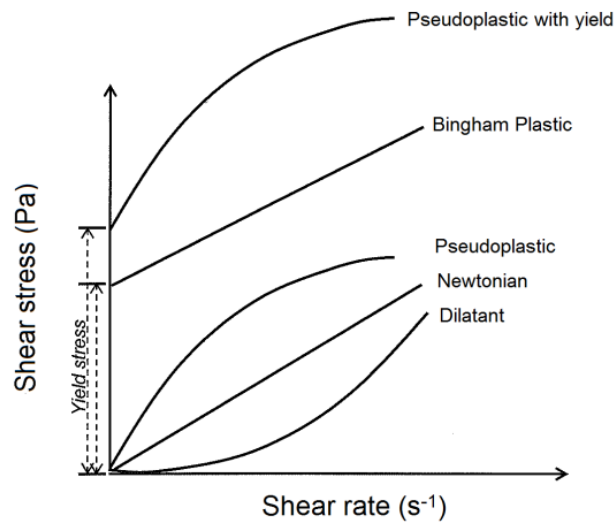


Figure 1. 1 Types of rheological behavior [68]

- **Non-newtonian behavior**

Fluids whose flow properties are affected by shear rate are called non-Newtonian fluids. A constant viscosity value cannot be mentioned for such fluids. As a result, the apparent viscosity of non-Newtonian fluids at a given shear rate is examined. Shear stress divided by shear rate yields apparent viscosity ($\eta_{app}=f(\dot{\gamma})=\tau/\dot{\gamma}$) [66].

- **Timeless non-newtonian behavior**

Pseudoplastic behavior: Viscosity seen in pseudoplastic or shear-thinning fluids decreases as the shear rate increases. As can be seen from Figure 1.1, in pseudoplastic fluids, the shear stress increases as the sliding speed increases. The apparent viscosity reduces as the shear rate rises, as seen in Figure 1.1. Shear-thinning (pseudoplastic) behavior is modeled by the power law.

Rheological properties are considered as important quality criteria for emulsion products such as mayonnaise, sauces, salad dressings, etc. Knowing the rheological properties helps to control the quality of these products during production, storage, and transportation [70]. The relationship between consumer preferences and the rheological properties of foods is an important part of rheology science. Therefore, it is necessary to have theoretical knowledge of its rheological aspects.

Since rheological properties are closely related to sensory quality, shelf life, and microstructural properties of salad dressings, they are important parameters in determining the general quality of salad dressing. Salad dressings show non-Newtonian, yield stress, and viscoelastic solid rheological properties [31]. Many researchers have studied the rheology of salad dressings because of the relevance of insufficient formulation selection, production conditions, and quality control. The fat content, on the other hand, influences the rheological and sensory properties of salad dressings, including mouthfeel, texture, and taste. Salad dressings are O/W emulsions with low-fat content. From this point of view, the salad dressings produced are expected to comply with all quality parameters required in emulsion products.

In salad dressing-type emulsion products, the rheological properties are significantly influenced by the ingredients used in the product preparation, especially the oil content. Especially as the emulsifier, stabilizer, and oil content increase, the consistency coefficient and storage modulus of the emulsion increase significantly. These components need to be optimized for the production of products with the desired structural properties [71]. However, emulsifiers have a lower influence on rheological characteristics than stabilizers and oil in salad dressing and mayonnaise [36].

Emulsifiers, as in salad dressings, also reduce the oil-water interface tension in structures consisting of oil-in-water emulsions. Thus, they play a critical role in ensuring emulsion stability. In terms of emulsion quality, the amounts of emulsifiers should be adjusted most appropriately and correctly. The amount of emulsifier must reach a concentration that can form a film around the oil particle. If the emulsifier concentration is below the desired level, an interaction begins between the oil particles. Due to this interaction, emulsion stability is decreased during storage. On the other hand, if the amount of emulsifier is used excessively, another flocculation mechanism (Depletion flocculation) comes into play, and emulsion stability decreases [72].

The primary role of stabilizers in salad dressings is to improve viscosity and other viscoelastic properties of the continuous phase by interacting with water in the

continuous phase. Accordingly, they increase the emulsion stability. Besides, stabilizers are used to determine the flow behavior properties of salad dressings, to improve their recovery properties, and to tighten the structure. In addition to these benefits, excessive use of stabilizers produces a significant rise in the viscosity of the continuous phase as well as degradation in the product's flow behavior, viscoelastic characteristics, and recovery capabilities. For this, the amount of stabilizer must be within certain ratios and limits. The most preferred stabilizers in products such as salad dressing are; xanthan gum, guar gum, locust bean gum, pectin (low ester), microparticulate proteins, starch (modified, unmodified, or pregelatinized), carrageenan, carboxymethyl cellulose, methylcellulose, gelatin, and agars.

Salad dressing includes at least 30% vegetable oil by weight. The fat content they contain affects their stability. High-fat salad dressings are very stable against flocculation (droplets coming together without breaking their integrity). When the oil ratio falls below 60-65%, their stability decreases. For emulsion products such as salad dressings, the main quality parameters have been determined. These are physicochemical, structural recovery, ionic charge balance (zeta potential), particle size, and emulsion stability [36, 73]. Most bacteria involved with food deterioration are inhibited by the acidic conditions and other variables (such as decreased water activity) present in salad dressings and mayonnaise. However, microbial deterioration of these products does occur from time to time as a result of the growth of a specific group of bacteria. These physicochemical and microbiological processes cause considerable sensory losses in oil-containing products like salad dressing and decrease the product's shelf life.

In products like mayonnaise and salad dressing, rheological tests such as flow behavior, oscillation, creep-recovery, and rheological tests such as creep recovery are applied to determine the emulsion structure and the interaction between the components that make up the emulsion [74, 75]. For example, the flow behavior rheological properties give an idea about the strength of colloidal interactions between particles in the emulsion, while the oscillation tests in the linear viscoelastic region and the 3-ITT test provide important insights in determining the reversible and irreversible structural deformation in the product in both linear

and non-linear viscoelastic regions [74, 75]. Also, the creep and recovery test demonstrates the product's recovery after a certain period of deformation in the linear viscoelastic region [76]. In this way, the effect of both the components in the product structure and the process conditions on the product is tested.

In a study, the impact of xanthan gum and starch addition on the rheological characteristics of 37.5% oil-reduced mayonnaise was examined and compared with full-fat mayonnaise. The reduced-fat mayonnaise containing 37.5% oil, 5.6% modified starch, and 0.1% xanthan gum exhibited a rheological behavior similar to full-fat mayonnaise-containing gum. Researchers also reported that xanthan gum provides a significant increase in yield stress and consistency coefficient in both products [77]. The result of this research shows that a small increase in the amount of xanthan gum can cause a significant change in the structure of the product. Therefore, the oil and gum ratio must be optimized in salad dressings.

1.1.6 Stability of salad dressing

The resistance of a food emulsion to changes in emulsion characteristics over time is referred to as emulsion stability. The higher the emulsion stability of a product, the slower the rate of change of the characteristic (emulsion) properties of the product according to the external environment conditions. The stability of emulsions can be deteriorated due to various physical and chemical processes. The concept of stability in salad dressings can also be divided into two groups as physical and chemical stability. Chemical stability is related to the reaction of oil droplets dispersed in water with dissolved oxygen and formation of oxidation products, while physical stability is the case of oil droplets leaving their stable distribution in the water phase over time, resulting in the separation of oil droplets in the emulsion phase and causing creaming [72]. In other words, while chemical stability results from changes in the chemical structure of molecules, physical stability results from changes in the structural organization or mechanical distribution of molecules.

- ✓ Physical instabilities occurring in emulsions;
 - Creaming
 - Flocculation
 - Coalescence
 - Phase Separation
 - Ostwald Maturation
- ✓ Chemical instabilities occurring in emulsions;
 - Oxidation
 - Hydrolysis

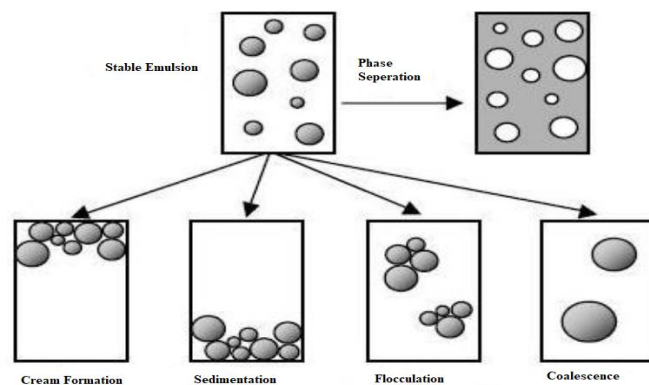


Figure 1. 2 Schematic view of destabilization observed in emulsion [78]

Significant quality losses occur in salad dressings that are physically or chemically changed during storage and their shelf life is reduced (Figure 1.2). These events occurring in emulsion products such as salad dressing vary according to the dispersion phase, continuous phase, and interface properties of the emulsion.

Salad dressing stability refers to a food emulsion's capacity to withstand physical and/or chemical processes that cause changes in its qualities. Physical changes often mean creaming, settling, agglomeration, coalescence, and phase reversal (Figure 1.2). The chemical processes in the food emulsion are determined by a series of chemical structure changes as a result of oxidation, lipolysis, proteolysis, or polymerization [36].

The stability of salad dressing and mayonnaise products, which usually have a shelf life of 9 to 12 months, is affected by the emulsifying capacity of various emulsifiers, the ratio of each component, homogenization methods, *pH*, size of dispersed droplets, viscosity, as well as the transport and storage conditions to which food emulsions are subjected.

This study will show that cold-pressed oil by-products are effective in improving the stability of low-fat salad dressings. In particular, the interaction between protein-polysaccharide and continuous phase and cold-pressed oil by-products affects emulsion stability. Due to the stabilizer properties of cold-pressed oil by-products, its water-holding properties are extremely high, and flocculation that may occur when the oil ratio is reduced can be prevented by the use of cold-pressed oil by-products, and with its stabilizer feature, the movement ability of the continuous phase can be limited and physical changes can be prevented.

1.1.7 Microbiological properties of salad dressing

Salad dressing and mayonnaise are examples of food items having a relatively stable structure. The majority are resistant to microbial spoilage and are degraded by only a few specific microorganisms. Lactobacilli, bacilli, and yeast are common organisms. The most common organisms in spoiled products are yeasts and, to a lesser extent, lactobacilli [79]. Disruptive organisms are organisms that can survive or grow at low *pH* values, high salinity, and/or high sugar concentrations of these products. In a study examining spoiled mayonnaise and salad dressings, Kurtzman, Rogers and Hesseltine [80] stated that 13 of 17 samples contained yeast which was recognized as *Saccharomyces bailii*. The main yeast species that can cause deterioration are *Zygosaccharomyces bailii*, *Z. rouxii*, and *Pichia membranifaciens* [81, 82]. Among the lactic acid bacteria, *Lactobacillus fructivorans* and *Lb. buchneri*. The deterioration in salad dressings is not in terms of health but the product quality.

The formulation of the aqueous phase plays the most important role in microbial stability in products such as salad dressing and mayonnaise. The water activity of salad dressing is 0.929 whereas the water activity of mayonnaise is 0.925. Salad dressings have a lower fat phase ratio compared to mayonnaise. Therefore, the

concentration of acetic acid and salt in the aqueous phase is less. This makes salad dressings more sensitive to microbial growth compared to mayonnaise. While the amount of acetic acid in salad dressings varies between 0.5-1.5%, the salt ratio of the aqueous phase varies between 1-4%, and the sugar ratio between 1-30%. The *pH* value of salad dressings is usually around 4. The low value, the acetic acid concentration is more than 0.5% and the aqueous phase having a certain salt concentration make salad dressings very stable against microbial growth. Since moderate heat treatment is applied in salad dressing products, a microbial safe product can be obtained by adjusting the value of the aqueous phase, acetic acid, and salt concentration at an appropriate level (International Commission on Microbiological Specifications for Foods (ICMSF), 2005).

The use of unpasteurized liquid egg and egg yolk in salad dressing formulation may pose a risk for *Salmonella* spp. pathogens. Therefore, the use of non-pasteurized liquid eggs and egg yolks is not recommended. Another important source of contamination is spices. It is recommended that the spices are preserved before use, such as irradiation. Apart from this, oil, salt, sugar, and antimicrobial components used in the formulation are not considered as sources of contamination.

Many factors such as the antimicrobial substance of salad dressings, low *pH* value, storage with mouth closed, acetic acid ratio close to 1% limit the growth of microorganisms. The most important antimicrobial preservative source for mayonnaise and salad dressing is acetic and/or citric acid. It has been reported that if the amount of acetic acid is 0.2% more in the aqueous phase, it is sufficient to control pathogenic microorganisms. Besides, when the acetic acid amount was 0.7% and the value was 4.5, a 4 log unit reduction was found in the number of *Salmonella* and *Listeria monocytogenes* [72].

Good manufacturing practices (GMP) should be followed to prevent products like salad dressing and mayonnaise against microbial deterioration. This necessitates thorough microbiological control of raw materials, crucial control points, as well as meticulous cleaning and sterilization of manufacturing tools and equipment.

1.1.8 Microstructure of salad dressing

Microstructural properties provide information about the structure, size and interaction, and integrity of the ingredients in food emulsions, which cannot be obtained with the naked eye. Therefore, information about the size and homogeneous distribution of oil particles can be obtained with the classical optical microscope.

Particle size in salad dressings prepared with cold-pressed oil by-products is important for the quality of salad dressings. Particle sizes can be controlled by emulsifier and homogenization process. In this sense, cold-pressed oil by-products can reduce particle sizes due to their emulsifier quality due to the proteins it contains, and at the same time, particle size control can be achieved with the homogenization process. Therefore, it is important in terms of determining the quality of salad dressings.

1.1.9 Latest approaches in the production of salad dressing

Salad dressing manufacturing has recently concentrated on products with low-fat and salt content, low-calorie content, and health-beneficial functional additives. The main idea behind making low-calorie sauce is to minimize the ratio of egg yolk powder to fat. The flaws in the structure of salad sauces that may be seen by lowering the fat ratio are reinforced by the addition of stabilizers, particularly xanthan gum. Proteins that provide comparable activities are chosen over high cholesterol emulsifiers like egg yolk [36]. The number of studies on the use of legume flours in salad dressing production is increasing [31, 38]. In addition to proteins, studies have been conducted to use polysaccharide components such as some pre-gelatinized starch in salad dressings with reduced-fat [40].

Another new approach in salad dressing production is the production of prebiotic and probiotic salad dressing. Especially inulin, pectin, and fibers obtained from some fruits can be used in the salad dressing preparation [36]. Some studies conducted for this purpose show that it is possible to produce prebiotic and probiotic salad dressing [71]. Another significant approach in the preparation of salad dressing is the use of raw materials containing functional components that are beneficial to health. In this perspective, we may assess the usage of cold-

pressed oils, olive oil, and oils containing polyunsaturated fatty acids in salad dressing preparation [36]. One of the most significant objectives of our research is to employ cold-pressed oils with health-promoting components in the preparation of salad dressing. In addition to probiotic microorganisms, several bacterial species that produce exopolysaccharides can be utilized in salad dressing preparation. Thus, the preparation of salad dressings containing exopolysaccharides instead of or in addition to stabilizers such as xanthan gum will be examined.

In salad dressing, the combined impacts of salt and oregano essential oil on the growth of *Escherichia coli* were investigated Cattelan, Nishiyama, Gonçalves and Coelho [83]. Even though essential oils have been indicated to have antimicrobial activities in vitro in several studies, little is known about their antimicrobial impacts in food items and interactions with food components [84]. In salad dressing samples, RSM was used to assess the individual and combined effects of oregano essential oil (OEO) and salt (sodium chloride) against *Escherichia coli* (ATCC 8739). As a result, the combination of OEO and salt-affected the bacterial count [84]. On the other hand, the adverse effect of salt was concluded with the highest NaCl concentrations and lowest bacterial count. When applied at inversely proportional concentrations, essential oil and salt exhibited antibacterial activity against the *E. coli* strain. As a consequence, given the conditions studied, the use of OEO combined with NaCl to reduce *E. coli* in salad dressing may be considered promising.

1.1.10 Low-fat salad dressing

Commercial salad dressing contains vegetable oil (not less than 30% by weight), egg yolk (4-6% wt), vinegar, thickening agents, salt, and flavoring material.

Oil, one of the main components, positively affected the final product's properties of rheological and sensory such as taste, texture, creamy texture, and shelf life [77]. As a type of oil-in-water emulsion, salad dressings are divided into three types according to fat content: full-fat (normal conventional oil level), reduced-fat (minimum 25% fat), and light (1/3 fat or semi-fat) [85]. The adverse health impacts of excessive eating of certain types of lipids prompted the development of

low-fat salad dressing processing in the food industry [77, 86, 87]. Overconsumption of fat is linked to some health issues, including obesity, hypertension, and cardiovascular disease [88]. Therefore, American Heart Association has recommended limiting fat consumption to less than 30% of total consumed calories [89]. The trend toward low-fat diets among consumers, as well as an increase in the consumption of salads and steamed vegetables, has raised the demand for low-fat salad dressings [88].

It can be challenging for the food industry to produce low-fat products that satisfy customers' expectations for fatty sensations while avoiding substantial changes in texture, rheological, and organoleptic qualities such as poor texture, taste, appearance, stability, and mouthfeel [90]. Oil substitutes with varied functionalities should be used to replace the oil in the fundamental recipe when making low-fat salad dressing to achieve the same quality attributes as the original full-fat salad dressing.

Fat replacers, which are widely used in foods, have bulking, gelling, mouthfeel improvement, moisturizing, stabilizing, thickener, and texture enhancing properties [91, 92]. The removal or decrease of fat in foods can cause some problems and it may be difficult to obtain the desired characteristics. To overcome these problems, it is necessary to use more than one oil substitute and to create appropriate combinations of them, and also to adjust the substances to be added to the formula and the process steps accordingly. Fat replacers are known to be of great variety. The biggest problem in the use of oil substitutes is that the brittleness, moistness, and lubricity given by the oil in the standard product cannot be achieved [93].

Fat mimetics: These are compounds that imitate the sensory or functional characteristics of triglycerides but do not replace fat on a gram-gram basis. They are carbohydrate-based, protein-based, or fat-based components with 0-9 kcal / g energy that can be utilized alone or in combination. By absorbing water, they provide the oil's lubrication, mouthfeel, and other properties. However, this additional water is not suitable for frying operations. Some of them can be used in the oven cooking. However, in the oven; They may be exposed to excessive

browning at high temperatures [94]. Fat mimetics are not used as a complete substitute for oil use. However, they mimic some of the properties provided by oil in food [95-97]. In the literature, there are lots of studies on low-fat salad dressings [31, 98-102].

Fat substitutes: They are components that can be used as a gram-for-gram replacement for oil because of their similarity to oils and fats. These are similar to triglycerides in both physical and chemical properties. Since they are oil-based, they are generally stable in cooking and frying temperatures [94]. Fat substitutes have the same functional and sensory qualities as fat in a foodstuff, but they often contain fewer calories than fat or no calories and can be used to substitute some or all of the fat normally found in a food product [95, 96].

Table 1. 1 Fat replacers in food applications [103]

Carbohydrate-based fat replacers	Foods	Protein-based fat replacers	Foods
Maltodextrins	Salad dressings, sauces, dairy products, spreads, frostings, fillings, processed meat, frozen desserts.	Micro-particulate protein	Soups, sauces, gravies, baked goods, cereal and grain products, cooking and salad oils, dairy products, refrigerated and frozen desserts,
Dextrins	Salad dressings, puddings, spreads, dairy-type products, frozen desserts.	Modified whey protein concentrate	Soups, sauces, gravies, baked foods, dairy products, refrigerated and frozen desserts
Gums	Salad dressings, sauces, bakery goods, soups, meats, yogurts, frozen desserts, meats.	Protein blends	Baked foods, dairy products, refrigerated and frozen desserts,
Polydextrose	Salad dressings, frozen dairy desserts, gelatins, puddings, baked goods, chewing gums.		
Fiber	Frozen reduced-fat bakery goods.		
Inulin	Whipped cream, cheese, yogurt, frozen desserts, baked goods, dairy products, fiber supplements.		
Carrageenan	Low-fat deserts, cheeses.		
Z-trim	Baked goods, cheese, ice cream.		

Fat-based fat mimetics provide emulsifying properties, mouthfeel, and retain flavor in reduced-fat salad dressings whereas carbohydrate-based fat mimetics provide to improve viscosity, mouthfeel, and texture properties and protein-based ones enhance texture and mouthfeel [28]. To fulfill mouthfeel sensations in reduced-fat O/W emulsions, many approaches for enhancing the rheological characteristics of low-fat emulsions should be explored. Therefore, the substitutes that do not have calories/are low in calories can replace the fat, which can provide the properties improved by the fat and do not change the taste have been searched for a long time. In the food industry, commercial fat replacers, which are carbohydrate-based, fat-based, and protein-based replacers, are used to provide the desired fatty mouthfeel without the calorie value [31]. As fat substitutes in low-fat salad dressing, cellulose derivatives, starch, gums, and oat and wheat bran can be utilized. Different fat mimetics can also be available to fulfill mouthfeel sensations in low-fat O/W emulsions [100, 104].

Table 1. 2 Fat mimetics used in food application [103]

Derived from proteins	Foods
Simplese	Salad dressing, mayonnaise, dips, sour cream, yogurt, cheese, baked products, frozen dessert products, frostings, margarine
LITA	
Trailblazer	
N-Flate	
Derived from carbohydrate	Foods
Gums: Guar, Xanthan, Locust bean, Carrageenan, Gum arabic, Pectins	Salad dressings, baked goods, sauces
Starches	Salad dressings, sauces, spreads, baked products, frostings, fillings, margarine
Microcrystalline cellulose	Salad dressings, sauces, frozen desserts, dairy products
Powdered cellulose	Baked products, frying
Methyl cellulose	Baked products, dry mix sauces, frozen desserts
Hydroxypropyl methylcellulose	Salad dressings, sauces
Maltodextrins	Salad dressings, sauces, imitation sour cream, baked products, frostings, processed meat, frozen desserts, table spreads, margarine
Polydextrose	Salad dressings, sweet sauces, fruit spreads, peanut spreads, baking products, frostings, frozen dairy desserts, puddings and fillings, hard and soft candy, toppings and syrups confections, chewing gum,
β - glucan	Baked products

1.2 The objective of the thesis

In recent years, consumer preference has concentrated on minimally processed foods such as salad with the increase in the quality of life. As a result of this situation, consumption of emulsion products like salad dressing and mayonnaise consumed with salad has increased significantly. A relationship has been established between these products and health concerns such as obesity, cardiovascular disease, and high cholesterol because of the high oil content of these food products. This negative situation reveals the necessity of developing some alternatives in the production of these products. Therefore, medium-fat, low-fat, and non-fat products have gained popularity with the global obesity disease, consumers' desire to eat fewer calories with increasing nutritional knowledge, and cardiovascular diseases caused by excessive intake of saturated fats. Although reducing oil in such emulsions has met the consumer preference in this respect, it is very difficult to produce products with the same structural properties in low-oil emulsions.

The by-products obtained by the cold-pressed oil technique have a significant potential use as a food source without high heat treatment (≥ 50 °C) and chemical treatment (e.g. hexane) and the food components in their structure remain retained. Therefore, there is less change in bioactive components compared to refined oils. Also, consumer preference has turned to cold-pressed oils. Seeds and kernels to be used in cold-pressed oil production are subjected to drying before production and the moisture content is reduced depending on the seed variety. In the by-products obtained after oil extraction, the dry matter mostly consists of proteins and polysaccharides. From this point of view, cold-pressed oil by-products can be utilized to enhance rheological properties in oil-reduced products, due to the high protein and polysaccharide content in the by-products. Cold-pressed oil by-products obtained from black cumin seeds, coconut, flaxseed, and pumpkin seed oil have a high potential for use in emulsion because of their structure-improving properties due to their high fiber and polysaccharide content and their oxidative stability-increasing properties due to their rich phenolic substance content. In this study, it is aimed to produce low-fat and functional salad dressing

by using cold-pressed black cumin, coconut, flaxseed, and pumpkin seed oil by-products.

The purpose of this research is to characterize the cold-pressed industry by-products (by-products of cold-pressed flaxseed, pumpkin seeds, black cumin seed, and coconut) and to examine their possible application in salad dressings as a natural stabilizer and fat replacement, antibacterial, and antioxidant agent. The physicochemical, biological, and antibacterial characteristics of several cold-pressed edible oil by-products will be studied, and the determination of the potential use of these by-products in the food industry. The characterization of steady shear, dynamic rheological, 3-ITT rheological properties, and emulsion stability (thermal loop test), as well as the zeta potential value, particle size, and oxidative stability of low-fat salad dressings stabilized by cold-pressed by-products, were also aimed.

1.3 Hypothesis

It is to produce low-fat salad dressing by using cold-pressed flaxseed, pumpkin seeds, black cumin seed, and coconut oil by-products to improve nutritional components, rheological properties, emulsion stability, and oxidative stability.

2.1 Material

In this research, cold-pressed oil industry by-products (the cold-pressed extraction of black cumin, flaxseed, pumpkin seed, and coconut) were used as the raw material supplied from ONEVA Food Co. (Istanbul, Turkey) (Figure 2.1). The harvest years of the seeds are 2018. These by-products are taken from the same serial production since the chemical composition of cold-pressed oils will be affected by conditions such as product and production differences. The cold pressing machine used in the study is a screw press, 710 mm in length and 260 mm in width, motor power is 1.5 kW, energy consumption is 400-850 Watt/hour, transmission has helical shaft gear features. It has a wide variety of seed processing features, including more than 200 seed processing capacity between 1-50 kg per hour. The capacity of the cold pressing machine is 1080 kg product/day. These by-products were delivered to Food Engineering laboratory in Yıldız Technical University and were kept at 4°C in a closed and opaque environment until analysis. These by-products were ground using a laboratory mill (PX-MFC 90 D, Kinematica, Malters, and Switzerland) and sieved through mesh No.140.

Sunflower oil, vinegar (i. e., the acidifying agent), and salt were purchased from a local market in İstanbul. The chemicals used in the analyzes were xanthan gum, sodium benzoate, egg yolk powder, phenol phthalene, methanol, ethanol, KOH, hexane, and potassium iodide were purchased from Sigma (St. Louis, USA) and Merck (Darmsdat, Germany). All standards were analytical grade (Merck, Darmstadt, Germany).



Figure 2. 1 Cold-pressed oil by-products obtained by cold pressing of coconut (1), pumpkin seed (2), black cumin (3), and flaxseed (4)

2.2 Methods

2.2.1 The physicochemical properties of by-products

2.2.1.1 Determination of dry matter

The dry matter of the by-products was analyzed quantitatively using the method given by AOAC International's approved methods of analysis [105]. The by-products of 10 ± 0.1 g are dried until the constant weight at 105°C in a hot air dryer (FN 120, Nuve, Ankara, Turkey). After the drying, weights were measured by an analytical balance (Sartorius $d=0.1$ mg Laboratoire HUMEAU). The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.2 Determination of ash content

The ash contents of the by-products were determined by incinerating in a muffle furnace (Barnstead Thermolyne 72700) at 550°C for at least 5 hours according to official analysis methods of AOAC International [106]. The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.3 Determination of oil content

A Soxhlet extraction method was preferred to assess the oil content of the by-products. For this analysis, 500 ml glass flasks were kept in the oven at 105 ± 2 °C for two hours and were maintained at an almost constant weight. After allowing them to reach room temperature in a desiccator, they were weighed. The by-products were milled to obtain the smallest possible particles to ensure the optimal penetration of the solvent into the oil in-house. The ground samples were weighed 10 g and placed first in coarse filter paper and then coarse filter paper was placed in the cartridge. The cartridge is covered with oil-free cotton to prevent the solvent from overflowing the sample. The cartridge was placed in the apparatus and the heating plates were adjusted to 60 °C by adding solvent (hexane used as a solvent). The analysis was continued for 6 hours, at which time the analysis was completed, the solvent was removed by distillation, and the flasks were incubated for 1 hour in an oven at 105 °C to ensure that the remaining solvent was completely removed from the oil. The flasks in the oven were cooled to room temperature in the desiccator and the final weighing was performed. The total oil content of the by-products was then calculated using the following equation (2.1):

$$Oil(\%) = \left(\frac{M_1 - M_2}{m} \right) \times 100 \quad (2.1)$$

M_1 = weight of oil + glass balloon (constant weighing)

M_2 = weight of glass balloon (constant weighing)

m = weight of the sample

The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.4 Determination of protein content

The protein contents of cold-pressed by-products were determined by the Kjeldahl method [107]. Approximately 3 g of the samples were weighed and placed in the

Khejdal flask. Then 25 mL of concentrated H₂SO₄ and 1 Khejdal tablet (potassium sulfate (K₂SO₄), sodium sulfate (Na₂SO₄), titanium oxide (TiO₂) and copper sulfate (CuSO₄) mixtures are added) and 200-250 °C for 2 h, It was allowed to burn for 8 h at 350-400 °C. The color of the burned specimens changed from black to a clear transparent color. After the samples were allowed to cool, 5 ml of 40% NaOH, 3 ml of 3% boric acid were added and distillation was initiated (Behr Distillation Unit-S5, Germany). After distillation for 1 min, titration with 0.1 N HCl was performed and the spent HCl was noted in ml. Total protein contents of by-products were calculated by multiplying by 6.25 (the obtained nitrogen values).

$$Nitrogen(\%) = \frac{(V_1 - V_0) * N * M}{m} * 100 \quad (2.2)$$

$$Protein(\%) = Nitrogen(\%) * 6.25 \quad (2.3)$$

where V₁= the volume of the HCl solution required for the blank test (mL), V₀= the volume of the HCl solution required for the determination (mL), N= the normality of used HCl solution used for the titrations (0.1 N), M= the molar mass of nitrogen (0.014 g/100 mol) and m= the mass of the sample (g). The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.5 Color measurement

A colorimeter (Konica Minolta, CR-400, Mississauga, ON, Canada) was used to measure the color parameters of the by-products. All measurements were made in the CIELAB system (L*, a*, and b*) and in triplicate at 25°C. The color values of the by-products were given as L* (whiteness (100)/darkness (0)), a* (red/greenness) and b* (yellow/blueness). The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.6 Browning index

Palombo, Gertler and Saguy [108] developed an enzymatic digestion procedure that included browning index analysis. According to this procedure, 1 gram of by-

products was weighed and put into 5 mL of distilled water at 45 °C, and 1.5 mL of the combination was added into test tubes containing 0.4 mL of pronase solution (Calbiochem 53702; 70,000 PUWg) per mL Tris buffer [109]. The test tubes were incubated at 45°C for 2 hours. After adding 0.15 mL of 100 % (w/v) TCA (trichloroacetic acid), the mixture was centrifuged at 4,700 rpm for 20 minutes. The materials were then filtered. The absorbance values were measured using a spectrophotometer (8453E UV-vis spectroscopy equipment and Agilent, USA) at 420 and 550 nm. The blank is distilled water, and the BI, which is calculated by Eq. (2.4) and expressed as optical density/g dry solids.

$$BI = A_{420} - A_{520} \quad (2.4)$$

where A_{420} and A_{550} are absorbance values of the samples measured at 420 and 550 nm, respectively. The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.7 Bulk density

The bulk density of the cold-pressed oil by-products was calculated using the method given by Al-Kahtani and Hassan [110]. The by-products weighed 20 g and were placed in a 100 mL graduated cylinder installed on the shaker chamber of a water bath. For 5 mins, the shaker was set at 100 revolutions per min. The bulk density of by-products was determined by dividing the powder's weight by the volume of the cylinder. The bulk density of by-products was expressed in grams per milliliter (g/ml). The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.8 Determination of water activity

The by-products were put into a water activity meter after being placed in plastic cups to cover the surface (AquaLab, 2.0, USA). The results were obtained at 25°C. The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.9 Determination of pH

The pH values of the by-products were measured by a pH meter (WTW-Inolab, Weilheim, Germany) in a suspension of 10% (w/v) powder of the by-products in distilled water at 25 °C. The pH meter probe was inserted into the suspensions and measured directly. The mean values and standard deviations were given for triplicate measurements in Table 3.1.

2.2.1.10 Determination of Volatile Composition

The volatile compounds in the by-products were examined using a gas chromatography–mass spectrometry (GC–MS) system (7890A GC system, Agilent) with a DB-WAX column (60×0.2501 m inner diameter, 0.25 mm film thickness) [111]. The oven temperature was set to 40 °C for 10 minutes, then increased to 110 °C at 3 °C/min, then to 150 °C at 4 °C/min, then to 210 °C at 10 °C/min, and lastly to 210 °C/min for 15 min. As a carrier gas, the flow rate of helium was fixed at 1.0 mL/min. The electron ionization detector voltage was 70 eV. Fibers adsorb the compounds at 40°C for 1 h before desorbing them from the injection port at 50°C for 15 min in splitless mode. By comparing retention indices and matching spectra with reference compounds in the data system, the volatile compounds were identified using GC–MS libraries (Flavor 2, NIST 05, and Wiley 7n). The peak areas were used to determine the volatile composition of the by-products as a percentage by dividing the area of each peak by the total area under all of the peaks.

2.2.2 The bioactive components of by-products

2.2.2.1 Extraction procedure

During the extraction process, 5 g of each of the by-products were extracted using 50 mL of the ethanol-water mixture (70:03 ethanol:water) at 25 °C. At 25 °C, the mixture was stirred by vortex before being shaken for 2 hours. After the extraction process, the solution was centrifuged at 4500 rpm for 5 min by a centrifuge (Universal 320, Hettich, Germany), and the supernatants were filtered with a 0.45

µm filter (Sartorius Stedim Biotech, Gottingen, Germany). For further investigation, the obtained extracts of the by-products were stored at -18 °C.

2.2.2.2 Determination of total phenolic content

Total phenolic contents (TPC) of by-products in methanolic extracts were determined by using a modified technique reported previously [112]. In the centrifuge tube, each of the extracts of the by-products (0.5 mL) was combined with 2.5 mL Folin-Ciocalteu reagent (0.2 N) and 2 mL of 2% (w/v) Na₂CO₃, respectively. The mixture was incubated in a dark place for 30 minutes at 25 °C. At the end of the period time, the absorbance of the solution was measured by a spectrophotometer (Shimadzu UV-1800, Japan) at 760 nm. TPCs of the by-products were given as mg of gallic acid equivalent (GAE) per 100 g.

2.2.2.3 Determination of antioxidant capacity (CUPRAC method)

The antioxidant capacity of methanolic extracts derived from by-products was used by the cupric reducing antioxidant capacity (CUPRAC) method, developed by [113]. Each extract of by-products (0.1 ml) was mixed with 1 ml of CuCl₂ solution (prepared as 170.48 mg CuCl₂·2H₂O/100 ml of water), 1 ml of Neocuproine (Nc) solution (prepared as 0.156 g Nc/100 ml of ethanol), and 1 ml of NH₄Ac (prepared as 7.708 g NH₄Ac/100 ml of water) solution, and then 1 ml of distilled water was added to the mixture. The absorbance of the extracts of by-products was measured at 450 nm by a spectrophotometer (Shimadzu UV-1800, Japan) after 60 min of incubation. The results were given as mg Trolox equivalent (TE) per 100 g [114].

2.2.2.4 Determination of total flavonoid contents

Total flavonoid contents (TFC) in the methanolic extracts of by-products were determined by using the method defined by Zhishen, Mengcheng and Jianming [115]. The each of extract (1 mL) was mixed with NaNO₂ (0.3 mL, 5%), AlCl₃ (0.3 mL, 10%) solution, and 4 mL distilled water. Then, 2 mL of NaOH (1 M) and 2.4 mL distilled water were added to the mix. A spectrophotometer (Shimadzu UV-1800, Japan) was used to measure TFC values at 510 nm. The results were given in mg of catechin (CE)/100 g.

2.2.2.5 Determination of phenolic profile

The phenolic profiles of by-products were determined using the modified method outlined by Ucar and Karadag [116]. The HPLC system used for an SPD-M20A diode array detection (DAD) analysis of extracts was Merck-Hitachi (LaChrom, Tokyo, Japan) equipment consisting of a DGU-20A5 degasser, an LC-20AT gradient pump, a SIL-20A autosampler, a CTO-10A5 VP column oven. The column was preferred as a Luna (5 μ m) C18 100A (250 \times 4.6 mm) (Phenomenex, Torrance, CA, USA). The temperature of the column will be adjusted to 25 °C. Mobile phase A was 2% (v/v) acetic acid in double-distilled water while eluent B was 0.5% acetic acid in double-distilled water and acetonitrile (50:50, v/v). Gradient elution is as follows: 10% B (0–2 min), 10–30% B (2–27 min), 30–90% B (27–50 min), and 90–100% B (51–60 min) and at 63 min reverts to initial conditions. The flow rate was adjusted as 1 mL/min. Chromatograms were recorded at 278, 320, and 360 nm.

Figure 2.2 showed that the individual phenolic of the by-products was determined by using 15 phenolic compound standards (caffeic acid, chlorogenic acid, chrysin, ferulic acid, gallic acid, kaempferol, myricetin, m-coumaric acid, o-coumaric acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid, quercetin, rutin, and syringic acid). Results were evaluated by integration area calculation; areas were calculated as the concentration from the calibration curves for each standard and given as mg/100 g sample for all by-product's extracts.

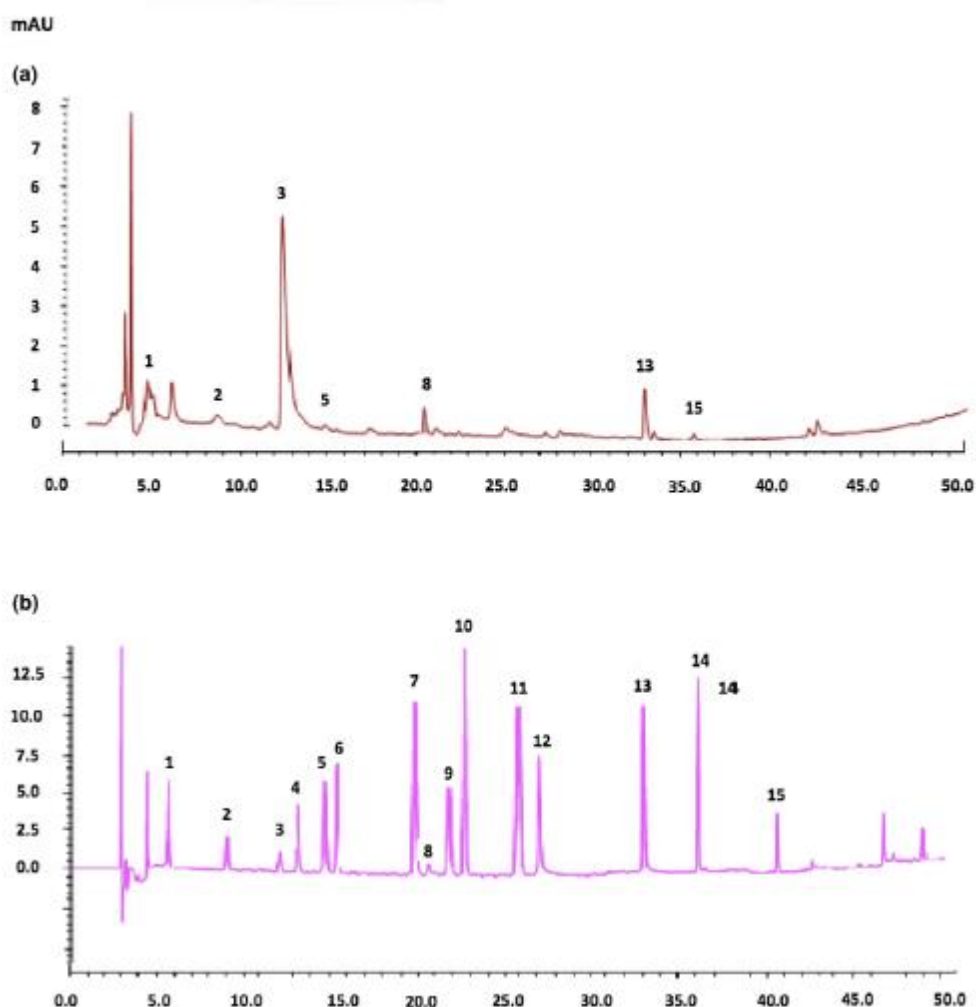


Figure 2. 2 HPLC chromatogram for the standard mixture¹

2.2.3 In-vitro digestion assay

The phenolic by-products in vitro digestion analysis were performed out using a previously established technique provided by [117]. Each by-product (5 g) was mixed with 20 mL distilled water. The method consists of two sequential steps. For the first step, 1.5 ml of pepsin solution (40 mg/ml) was added. Then, the pH was adjusted to 1.7 with an aqueous solution of HCl (prepared as 18.23 g/100 ml of water) to simulate gastric conditions. This mixture was shaken at 100 rpm at 37 °C in a water bath for 2 h. After 2 h, 2 mL of aliquots of postgastric digestion (Pg) were removed and kept at –20 °C. For the second step, 4.5 mL of 4 mg/mL

¹ 1: gallic acid; 2: protocatechuic acid; 3: chlorogenic acid; 4: p-hydroxybenzoic acid; 5: caffeic acid; 6: syringic acid; 7: p-coumaric acid; 8: elagic acid; 9: ferulic acid; 10: m-coumaric acid; 11: o-coumaric acid; 12: myricetin; 13: quercetin; 14: kaempferol; 15: chrysin.) (A and B shows POB and standard chromatograms, respectively.

pancreatin and 25 mg/mL bile salts combination was mixed in a 250 mL glass beaker. A sufficient solution of NaHCO_3 (2 g/100 mL water) was added to a cellulose dialysis tube to neutralize the sample's titratable acidity. The mixture was kept at 37 °C for 2 hours to simulate small intestine conditions. After the incubation step, the solution outside the dialysis tube was taken as the OUT sample, representing material that remains in the gastrointestinal tract. The solution that entered the dialysis tubing was taken as the IN sample, representing the material that entered the serum. Then, TPC was performed for the Pg and the postgastric IN and OUT samples. The percentage recovery of the bioaccessibility of phenolics was calculated by dividing the values determined for the IN fraction from the values determined for the initial (before digestion) values and then multiplying by 100 [118].

2.2.4 Antimicrobial activity of by-products

The antimicrobial activities of the extracts of by-products were determined by the agar diffusion well method. Therefore, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, and *Listeria monocytogenes* ATCC 13932 were selected as test pathogens. The pathogen cultures were cultivated overnight and then spread onto Petri dishes containing nutrient agar. After bacterial penetration, 3 wells were made on each plate using a cork borer. Inoculated Petri plates were incubated at 37°C for 24 h after adding 20 μL of extracts to each well individually, the ethanol:water (70:30 v/v) mix was used as a control. The inhibition zones were expressed in millimeters (mm).

2.2.5 The effects of by-products on salad dressing

2.2.5.1 Experimental design

Response surface methodology (RSM) and full factorial central composite design (CCD) were selected to assess the influence of different formulations of XG (%), oil (%), and by-product (%) to produce the low-fat salad dressing samples. As shown in Table 3.7, 17 different experimental points were obtained by utilizing Design Expert Software (Version 7; Stat-Easy Co., Minneapolis, MN) to identify the optimum formulation. The design included three factorial points for error

estimates. All points were analyzed in triplicate, and the results were given as mean values and standard deviations.

RSM and CCD were performed to determine the effect of different formulations for XG (%), oil (%), and FOB (%) to prepare the low-fat salad dressing. 17 different experimental points were obtained by using Design Expert Software (Version 7; Stat-Easy Co., Minneapolis, MN) to find the optimum conditions. For the estimation of the error, the design comprised three of the factorial points. The rheological properties of commercially produced salad dressing were taken into consideration in the selection of FOB, XG, and oil ratios. These values were taken into consideration in the selection of the FOB, XG, and oil ratios corresponding to K values. The K, K' and K'' were response variables, and FOB, XG, and oil were process factors. A quadratic model was fitted to the experimental data for each response. Model applicability was determined based on the R^2 , R^2 -adj, lack of fit, F, and P- values obtained from ANOVA. The optimization was performed based on the highest desirability value. The formulation, including the lowest oil content with a desirability value of 1, was chosen as the optimum formulation. Three central points were used. Analysis of all points was conducted in triplicate, and the results were reported as mean values and standard deviations.

The effect of different formulations of XG (g/100 g), oil (g/100 g), and POB (g/100 g) on the preparation of the reduced-fat salad dressing was determined according to RSM and CCD. As presented in Table 3.11, 17 different experimental points were determined by Design Expert Software (Version 7; Stat-Easy Co., Minneapolis, MN, USA) to obtain the optimum formulation. The design comprised three of the factorial points, for the estimation of the error. Model applicability was determined based on the R^2 , R^2 -adj, lack of fit, F, and P-values obtained from ANOVA. Based on the highest desirability value, the optimization was carried out.

The formulation of the lowest fat content based on the desirability value of 1 is accepted as the optimum formulation. This formulation contains three central points and analysis of all points were conducted in triplicate. Results are generated in mean values and standard deviations.

2.2.5.2 The determination of by-products contents for the effects on rheological properties of the low fat- salad dressing

Two different control samples which are the standard high-fat salad dressing (C1) and the low-fat control (C2), contained 30% and 10% oil content, respectively. The salad dressing formulated with 10% oil, and 1-5% by-products were prepared for a preliminary test. Rheological properties of the salad dressing formulated with by-products were compared to C1 and C2.

2.2.5.3 Salad dressing preparation

The by-products-enriched salad dressings were prepared by using the following ingredients which are XG, EYP, by-products (BOB, COB, FOB, and POB), and oil expressed as g/100 g. All formulations of the salad dressing were prepared according to the experimental design. The method of the preparation was as follows: the product was slowly added to water to form by-product– water dispersion and then the dispersion heated to 80 °C for 20 min to obtain complete hydration of by-product in water. After cooling the solution to 25 °C, XG and EYP were dissolved in the dispersion. The stirring of the mix was continued at 1000 rpm in a magnetic stirrer for 6 h to complete the hydration of the XG. Finally, an Ultra-Turrax digital homogenizer (Daihan, HG-15D, Gangwondo, Korea) was used for the preparation of O/W emulsions at 10,000 rpm for 3 min [119]. Finally, the salad dressing samples were obtained and pasteurized at 65 °C for 10 min and poured into brown bottles, and cooled to room temperature. In this study, all material (beakers, brown bottles, and probes) was used after the sterilization at 121 °C for 15 min. High fat (C1, 30% oil) and low-fat control salad dressing samples (C2, 10% oil) were prepared. XG and EYP were added as 0.35% and 3% in both control samples, respectively. A grape vinegar containing 4% acetic acid at the rate of 7.5% of the aqueous phase was used for the acidification of the products.



Figure 2. 3 Salad dressing samples²

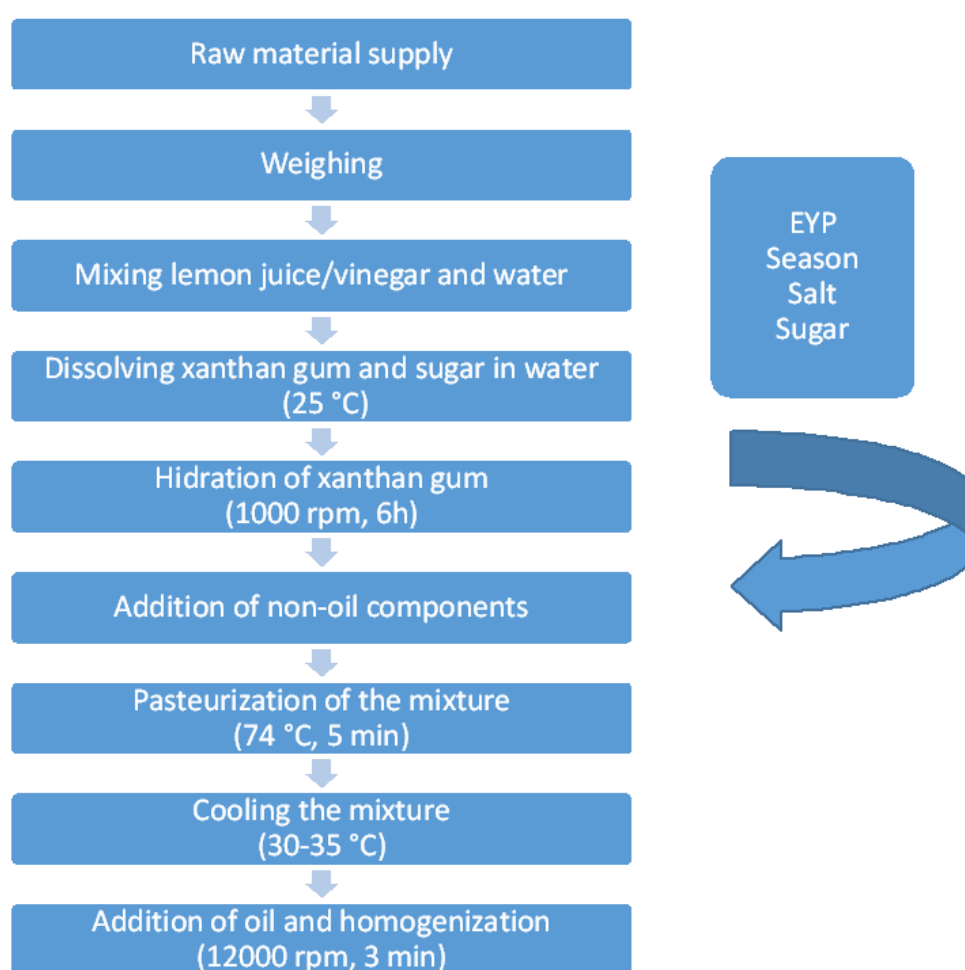


Figure 2. 4 Salad dressing production flow chart³

² C1: control sample with 30% oil, C2: control sample with 10% oil, BOB, black cumin oil by-product; COB, coconut oil by-product; FOB, flaxseed oil by-product; POB, pumpkin seed oil by-product.

³ EYP: egg yolk powder

2.2.5.4 Rheological analysis

The salad dressings' rheological characteristics (flow behavior, dynamic rheological, and 3-ITT) were determined at 25°C by stress or strain and temperature-controlled rheometer (Anton Paar, MCR 302, Austria) fitted with a Peltier system. PP50 probe (diameter: 50 mm) was utilized as measurement geometry and a 0.5 mm gap height was preferred for measurements. All rheological studies were conducted in triplicate at 25°C. Before starting the test, all salad dressings were reached to equilibrium at 25°C for 1 min.

The flow behavior in the rheological characteristics of salad dressings was investigated at shear rates (from 0.01 s⁻¹ to 100 s⁻¹). The shear stress and apparent viscosity parameters were measured. The experimental flow curves were fitted to the Power-law model expressed in Eq. (2.5), and the relevant parameters of steady shear rheological characteristics, K and n value, were determined

$$\tau = K \gamma^n \quad (2.5)$$

where τ was shear stress (Pa), K was consistency index (Pa s⁻ⁿ), γ was shear rate (s⁻¹), and n was the flow behavior index.

For the dynamic rheological analysis, a frequency sweep test was conducted. Before the oscillatory measurements, the linear viscoelastic region (LVR) was identified with an amplitude (stress) sweep test in a strain value (0.1%). In LVR, the frequency sweep test was performed in the angular velocity range (ω : 0.1–64 s⁻¹). In response to the rotational velocity, the storage (G') and loss modulus (G'') values were measured. The Power-law model parameters and nonlinear regression were used to compute the dynamic rheological parameters (G' and G'') [120];

$$G' = K'(\omega)^{n'} \quad (2.6)$$

$$G'' = K''(\omega)^{n''} \quad (2.7)$$

where G' (Pa) is the storage modulus, G'' (Pa) is the loss modulus, ω is the angular velocity value (s⁻¹), and K' & K'' are consistency index values, and n' & n'' represent the flow behavior index values.

3-ITT was applied to the salad dressing samples to determine the degree of recovery following deformation. The 3-ITT rheological properties of the salad dressings were determined at the constant shear rate (0.5 s^{-1}) and a variable shear rate (150 s^{-1}). The LVRs of the salad dressings were considered when the values were chosen, and the LVRs of the salad dressings stops at 50 s^{-1} . In the first time interval, the samples were exposed to 100s of shear at a relatively low shear rate of 0.5 s^{-1} . The shear rate was applied to 150 s^{-1} for 40 s in the second period. The dynamic rheological behavior in the second time interval was investigated in the third time interval by subjecting the samples to a modest shear rate in the first period. The change in viscoelastic solid structure (G') of salad dressing samples was studied for this purpose. In the third period, the behavior of the salad dressing samples was modeled using a second-order structural kinetic model (2.8):

$$\left[\frac{G' - G_e}{G_0 - G_e} \right]^{1-n} = (n-1)kt + 1 \quad (2.8)$$

where G' is the storage module in (Pa), k is the thixotropic rate constant, G_0 is an initial storage modulus (Pa) in the third time interval, and G_e is the equilibrium storage modulus (product completely recovers itself) .

2.2.5.5 Zeta potential, particle size, and polydisperse index

The zeta potential value, the particle size, and the polydisperse index of the salad dressings were determined by a zeta potential and particle size meter (Nanosizer, Malvern Instruments, Worcestershire, UK) and characterized by the dynamic light scattering (DLS) technique. Before the analysis, all samples were diluted 500 times with distilled water and homogenized in an ultrasonic bath for 5 minutes. For the zeta potential and the particle size measurement, 3 parallel measurements were performed, and the mean and standard deviation of the values were given.

2.2.5.6 Emulsion stability index

Visual analysis was used to measure the emulsion stability index (ESI) of salad dressings throughout a 28-day storage period. After 28 days, phase separation was observed in salad dressing. The height of the oil on the top of the salad dressings was measured. Eq. (2.9) was used to calculate ESI:

$$ESI = \frac{O_H}{E_H} \times 100 \quad (2.9)$$

where, O_H and E_H are the height of the oil phase and the height of total emulsion in cm, respectively.

2.2.6 Emulsion stability (Thermal Loop Test)

The thermal loop test, as a new approach, was initially used at high and low temperatures to demonstrate that the physical stability of oil in water emulsions may be determined [121]. Emulsions with different levels of stability were produced utilizing different XG concentrations ranging from 0.1 to 0.5 g/100 g in the recipe.

In this study, the thermal stability of salad dressings was determined by a thermal loop test. This test is a suitable and fast method for determining the emulsion stability of salad dressings in a short time by detecting structural changes and simulating temperature fluctuations during processing, production, storage, and transportation.

Salad dressings were treated through different numbers of thermal cycles. The change of modules from cycle to cycle expresses structural or morphological changes because of heat stress. Samples were exposed to 11 heat cycles ranging from 23 to 45 °C in the high-temperature stability test. The strain value and angular frequency (ω) were set at 10 Hz and 0.5 percent, respectively. The heating and cooling speeds were changed by 11 °C/min (Rheoplus for MCR 301). To investigate the thermal stability of salad dressing, the relative structural change value (Δ) was determined for G^* values by dividing the highest value of each cycle by the value of the first cycle with Eq. (2.10).

$$\Delta = \frac{G'_{\max}(G''_{\max}), i}{G'(G'')} \quad (2.10)$$

2.2.7 Oxidative stability (OXITEST)

The oxidative stability of the salad dressing (control samples and the optimum samples) was measured by OXITEST (Velp Scientifica, Usmate, MB, Italy) during the storage period according to Aksoy, Tekin-Cakmak, Karasu and Aksoy [122].

Approximately, 10 g of salad dressing was put into the sample cells. The temperature of the device was adjusted to 80, 90, 100, and 110 °C, and the oxygen pressure was adjusted to 6 bar. The induction period (IP) value was measured by the OXITEST to evaluate the oxidative stability values of the salad dressings. In addition, depending on the period, data on pressure change was received from OXITEST. The impact of temperature on the oxidation rate constant (k), which is the inverse of IP, was determined using the Arrhenius equation (2.11):

$$k = A_0 \times \exp\left(\frac{-E_a}{RT}\right) \quad (2.11)$$

where E_a represents the activation energy (kJ/mol), R is the ideal gas constant (8,314 J/molK) and the temperature is defined as T (K).

Activation enthalpy (ΔH^{++}), and entropy (ΔS^{++}) values were determined by using Eq. (2.12) derived from the activated complex theory:

$$k = \frac{k_B t}{h} \exp\left(-\frac{\Delta H^{++}}{RT} + \frac{\Delta S^{++}}{R}\right) \quad (2.12)$$

In Eq. (2.12), k_B is the Boltzmann constant ($1.3806488 \times 10^{-23}$ J/K), h shows the Plank constant (6.6261×10^{-34} J/s), T represents the absolute temperature (K), R is the ideal gas constant (8,314 J/mol.K), (ΔH^{++}) is the change in enthalpy (kJ/mol) and (ΔS^{++}) is the entropy change (J/mol/K). The required parameters were determined by the nonlinear regression model with Eq. (2.12). For non-linear regression, the Statistica software (StatSoft, Tulsa, USA) was utilized.

2.2.8 Optical microscopy

Under a light microscope with a digital camera (Cannon, Japan), salad dressings (control samples and optimal samples) were observed. A special software tool was used to examine the images. On a glass slide, 0.01 ml salad dressings were deposited and covered with a coverslip. Samples were examined at 26°C at 100 magnification after 30 min of equilibration.

2.2.9 Statistical analysis

All data were reported as a mean of three measurements with the standard deviation for each sample. The differences between the samples were assessed

using a one-way analysis of variance (ANOVA) combined with Duncan's multiple comparison test at $p < 0.05$ significance level.

The physicochemical and bioactive contents of all by-product analyzes were measured in at least 3 parallels. The statistical difference between the mean values of the samples was evaluated.

In the first stage, the statistical differences between the rheological properties of the salad dressing samples produced with 4 different by-products in the salad dressing samples were evaluated. Low-fat salad dressing samples produced with by-products were compared with low-fat and full-fat control salad dressing samples. All salad dressing samples were produced with at least 3 replications. At least 5 parallel measurements were taken from each replication for rheological analysis. The experimental design obtained from the Design expert program was used from the by-product of flaxseed and pumpkin seeds. The Statistica software program (Stat Soft Inc., Tulsa, UK) was used for statistical applications. The mean values of the parameters were compared using Duncan's multiple comparison tests ($p < 0.05$). Power-law model parameters were generated using nonlinear regression analysis as a consequence of the applied rheological study. Also, the mean values of the rheological analysis, zeta potential, particle size, and oxidative stability were determined. Nonlinear regression analysis was performed using the Statistica software package (Stat Soft Inc., Tulsa, UK).

RSM and CCD were performed to determine the effect of different formulations for XG (%), oil (%), and FOB (%) to prepare the low-fat salad dressing. 17 different experimental points were obtained by using Design Expert Software (Version 7; Stat-Easy Co., Minneapolis, MN) to find the optimum conditions. For the estimation of the error, the design comprised three of the factorial points. The rheological properties of commercially produced salad dressing were taken into consideration in the selection of FOB, XG, and oil ratios. These values were taken into consideration in the selection of the FOB, XG, and oil ratios corresponding to K values. The K, K' and K'' were response variables, and FOB, XG, and oil were process factors.

Also, the effect of different formulations of XG (g/100 g), oil (g/100 g), and POB (g/100 g) on the preparation of the reduced-fat salad dressing samples was determined according to RSM and CCD. As presented in Table 3.11, 17 different experimental points were obtained by using Design Expert Software (Version 7; Stat-Easy Co., Minneapolis, MN, USA) to determine the optimum formulation. The design comprised three of the factorial points, for the estimation of the error. A quadratic model was fitted to the experimental data for each response. Model applicability was determined based on the R^2 , R^2 -adj, lack of fit, F, and P- values obtained from ANOVA. The optimization was performed based on the highest desirability value. The formulation, including the lowest oil content with a desirability value of 1, was chosen as the optimum formulation. Three central points were used. Analysis of all points was conducted in triplicate, and the results were reported as mean values and standard deviations.

In this study, the potential use of cold-pressed black cumin seeds (BOB), coconut (COB), flaxseed (FOB), and pumpkin seed oil by-products (POB) as functional ingredients and natural oil substitutes in low-fat salad dressing were investigated. In the first stage of the study, by-product characterization was carried out. For this purpose, physicochemical and bioactive properties of by-products such as color, protein, oil and dry matter contents, antioxidant and antimicrobial activities, volatile compounds, phenolic profiles, fatty acid profiles, and in-vitro bioaccessibility of phenolic compounds were investigated. Flow behavior, dynamic rheological and 3-ITT rheological properties, and zeta potential values of low-fat salad dressings stabilized with these by-products were determined to investigate the potential of using by-products as a fat replacer. In the second stage of the study, the rheological properties (flow behavior, dynamics, and 3-ITT) of low-fat salad dressing samples obtained from these by-products were analyzed to determine the possible use of by-products (BOB, COB, FOB, and POB) as fat replacers in reduced-fat salad dressings. Therefore, salad dressing samples containing 1-5% by-products were prepared and these salad dressing samples were compared with low-fat and high-fat salad dressing samples in terms of rheological properties. In the third stage of the study, since FOB and POB samples could not reach the rheological values of 1% high-fat salad dressing samples, formulation optimization was performed to determine the optimum amount of these by-products. For this purpose, 17 different formulations were prepared with 1.0-5.0% POB and 0.5-1.5% FOB. Optimum FOB and POB amounts were determined by RSM and CCD. The optimum amount of FOP and POP was determined as 1.49% and 3.04%, respectively. In the final stage, high-fat salad dressing (HF-SD), low-fat salad dressing (LF-SD), low-fat salad dressing enriched with BOB (BOBLF-SD), low-fat salad dressing enriched with COB (COBLF-SD), low-fat salad dressing enriched with FOB (FOBLF-SD) and POB-enriched low-fat salad dressing (POBLF-SD) were prepared. Then, the emulsion stability (thermal

loop test), zeta potential and particle size, microstructure, and oxidative stability (OXITEST) of the samples were investigated.

3.1 The characterization of the cold-pressed oil by-products

Table 3.1 indicated the physicochemical properties of the by-products obtained from cold-pressed oil (BOB, COB, FOB, and POB).

Table 3. 1 Physicochemical properties of BOB, COB, FOB, and POB

Physicochemical Properties	BOB	COB	FOB	POB
Dry Matter (%, w/w)	89.7±2.31 ^a	92.1±0.04 ^a	88.6±1.37 ^a	89.1±0.94 ^a
Carbohydrate (%, w/w)	37.56±0.50 ^c	60.82±1.31 ^a	46.37±1.77 ^b	32.26±0.55 ^c
Protein Content (%, w/w)	27.24±2.15 ^c	16.36±1.77 ^d	27.67±1.55 ^b	44.02±2.50 ^a
Oil Content (%, w/w)	19.38±0.77 ^a	11.79±1.01 ^b	9.15±1.27 ^c	9.04±1.03 ^d
Ash Content (%, w/w)	3.30±0.00 ^d	2.93±0.00 ^c	2.87±0.00 ^b	2.48±0.00 ^a
a_w	0.42±0.00 ^c	0.37±0.00 ^d	0.49±0.01 ^a	0.45±0.01 ^b
L*	38.22±0.83 ^c	67.71±0.82 ^a	43.39±0.39 ^b	43.98±0.65 ^b
a*	2.45±0.01 ^b	5.82±0.29 ^a	5.92±0.34 ^a	-3.52±0.26 ^c
b*	-2.97±0.43 ^d	22.47±0.08 ^a	3.25±0.23 ^c	10.58±0.30 ^b
BI	0.71±0.001 ^d	1.76±0.013 ^a	1.28±0.004 ^c	1.45±0.002 ^b
Bulk density	1.50±0.00 ^b	1.41±0.01 ^c	1.27±0.00 ^d	1.55±0.00 ^a
pH	5.77±0.01 ^b	5.67±0.00 ^b	6.21±0.02 ^a	6.02±0.00 ^c

¹aw: water activity; BI: browning index; BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods (p < 0.05).

3.1.1 Dry matter and ash contents

Dry matter and ash contents of the by-products were found to be very similar to each other, ranging from 88.6% to 92.1% and 2.48% to 3.30%, respectively. Before the cold-pressed processing, the product moisture was lowered to the optimal level to maximize the oil extraction yield. The highest ash content was found in BOB, followed by COB, FOB, and POB. In a study, the dry matter contents of cold-pressed oil by-products from walnut, pomegranate seed, grape seed, almond were determined between 90.55 and 93.71% while the ash contents of these by-products were between 3.07% and 5.72% [45]. It can be revealed from this study that the by-products had a high degree of dry matter material.

3.1.2 Protein Content

Table 3.1 showed that the cold-pressed oil by-products could be regarded as high-quality protein and carbohydrate sources. Protein, oil, and carbohydrate contents ranged from 16.06% to 44.02%, 9.04% to 19.38%, and 32.26% to 60.82%, respectively (Table 3.1). The protein, oil, and carbohydrate compositions of the by-products differed significantly depending on the sample type ($P<0.05$). POB had the highest protein content, followed by BOB, FOB, and COB. As can be seen from the findings, all by-products contain large quantities of protein, suggesting that they may be utilized to boost the protein content of various foods. The protein contents of cold-pressed oil by-products from walnut, pomegranate seed, grape seed, almond were between 9.38% and 34.06% [45]. Çam, İçyer and Erdoğan [123] fortified ice cream with cold-pressed oil by-products and found that the enriched ice cream samples possessed higher protein content than the control sample.

3.1.3 Oil Content

Oil contents of the by-products varied between 9.04% and 19.38 %. Except for FOB and POB, the other by-products possessed higher than 10% oil content, showing a low cold pressing yield, which is a common concern in the cold-pressed oil extraction industry. The high oil content would cause oil oxidation that would be a problem either in the storage of by-products or in the foods enriched by the by-products. Despite that, the oxidation may be prevented through the high

phenolic content of the samples. On the other hand, one of the most significant considerations in assessing the rheological properties of salad dressings is the quantity of oil used. The viscoelastic property decreased as the oil ratio decreased, resulting in a less compact structure [31, 36]. This lower flexible structure is the most significant problem during the manufacture of low-fat salad dressing. Protein and polysaccharides can contribute significantly to the viscosity of the continuous phase by holding with water molecules [31]. Therefore, cold-pressed oil by-products can be used in the enrichment of low-fat salad dressing due to their high protein and carbohydrate content.

3.1.4 Color value

The visual acceptance of cold-pressed oil by-products can be largely determined by the color values. The color values of the cold-pressed oil by-products depend on the samples and their color values change as the variety of by-products changes (Table 3.1). L^* , a^* , and b^* values of POB, FOB, COB, and BOB were ranged from 38.22 to 67.71, from -3.52 to 5.92, from -2.97 to 22.47, respectively. The color values of the POB, FOB, and BOB samples were very comparable; on the other hand, the color values of COB, particularly L^* and b^* , were very different from the POB, FOB, and BOB samples. COB has the highest L^* value (the lightest color value) and the highest b value (the highest yellowness). According to Karaman, Karasu, Tornuk, Toker, Gecgel, Sagdic, Ozcan and Gül [45], different color values for walnut seed oil by-product, pomegranate seed oil by-product, grape seed oil by-product, almond seed oil by-product were obtained. The color of the by-products is also due to pigments (e.g. carotenoids) and phenolic pigments (e.g. anthocyanins, flavonols, and proanthocyanins). The color of the food products is one of the most significant variables affecting customer decisions. The color characteristics of foods can be altered with the addition of various additives. In this regard, these oil by-products can be used as color agents for food production.

3.1.5 Browning index

The BI values of the POB, FOB, COB, and BOB changed ranged from 0.71 to 1.76 and COB possessed the largest BI value (1.76) while BOB had the lowest value. Browning of by-products may be facilitated by the formation of Maillard-type

reaction products or the release of phospholipids that would facilitate the oil oxidation [124], and the formation of furfural derivatives [125].

3.1.6 pH value

The pH values of the POB, FOB, COB and BOB varied between 5.67 and 6.21. Karaman, Karasu, Tornuk, Toker, Gecgel, Sagdic, Ozcan and Gül [45] determined the pH values of the various cold-pressed oil by-products obtained from walnut, pomegranate, grape, and almond as 5.52, 4.43, 4.35, and 6.14, respectively.

3.1.7 Bulk density

The bulk density of the POB, FOB, COB, and BOB was found between 1.27 and 1.55. The bulk density values are also important in this respect because the flours of these by-products can be used for different purposes in foods. The lowest bulk density value of FOB among the samples indicates that it can be compressed more easily than the others.

3.1.8 Water activity (A_w)

a_w is an indicator of microbiological stability and an a_w value below 0.7 is expected to prevent microbial growth in food products. The a_w values of the POB, FOB, COB and BOB ranged from 0.37 to 0.49.

3.1.9 Volatile compounds

Table 3.2 showed that volatile compounds were identified for the by-products. As can be seen from Table 3.2, Ethanol (CAS) Ethyl alcohol was found as the predominant volatile compound of BOB (area:83.73% & height:77.91%), COB (area:58.51% & height: 64.45%), FOB (area:97.39% & height: 85.54%), and POB (area: 96.63% & height: 84.63%).

Table 3. 2 Major Volatile Compounds of BOB, COB, FOB, and POB

By-products	Compound	Area (A)	A (%)	Height (H)	H (%)	A/H
BOB	Oxirane (CAS)	732,397	0.24	125,659	0.31	5.83
	Epoxyethane					
	Ethanol (CAS)	258,897,316	83.73	31,463,720	77.9	8.23
	Ethyl alcohol					
	2-Propanol, 2-methyl- (CAS) tert-Butyl alcohol	6,418,956	2.08	5,365,093	13.3	1.20
	n-Hexadecanoic acid	20,303,174	6.57	1,703,326	4.22	33.49
	Hexadecanoic acid, ethyl ester (CAS)	22,804,634	7.38	1,726,268	4.27	13.21
	Ethyl palmitate					
COB	Ethanol (CAS)	255,359,771	58.51	31,101,254	64.5	8.21
	Ethyl alcohol					
	2-Propanol, 2-methyl- (CAS) tert-Butyl alcohol	6,329,508	1.45	5,494,630	11.4	1.15
	9-Octadecenoic acid (Z)- (CAS)	24,109,130	3.15	1,999,366	4.15	24.69
	Oleic acid					
	9-Tetradecenal, (Z)- 1,13-Tetradecadiene	5,417,673	1.24	837,172	1.74	6.47
	Ethyl linoleate	90,733,938	20.78	4,888,836	12.03	44.10
	Ethyl Oleate	54,529,292	12.50	3,012,515	6.24	32.21
FOB	1,1'-bibicyclo(2.2.2)octyl-4-carboxylic acid	173163	0.07	28656	0.08	6.04
	Ethanol (CAS)	241128460	97.39	31135200	85.5	7.74
	Ethyl alcohol					
	Methane, nitroso- (CAS)	320647	0.13	167139	0.46	1.92
	Nitrosomethane					
	2-Propanol, 2-methyl- (CAS) tert-Butyl alcohol	5975344	2.41	5068550	13.9	1.18
POB	Trans-Beta-Ionon-5,6-Epoxyde	172272	0.06	25258	0.07	6.82
	Ethanol (CAS)	270344610	96.63	31489294	84.6	8.59
	Ethyl alcohol					
	2-Propanol, 2-methyl- (CAS) tert-Butyl alcohol	6554135	2.34	5474155	14.7	1.20
	Octadecamethylcyc lononasiloxane	2720699	0.97	220084	0.59	12.36

¹BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product.

3.2 The bioactive compounds of the cold-pressed oil by-products

Table 3.3, Table 3.4, and Table 3.5 indicated the total phenolic content (TPC), antioxidant capacity (CUPRAC), total flavonoid content, individual phenolic profile, and bioaccessibility of TPC of by-products. Phenolic compounds, known as natural free radical scavengers, positively affect the sensory properties and oxidative stability of cold-pressed oil by-products.

3.2.1 The total phenolic content (TPC) and total flavonoid content (TFC)

Table 3.3 showed that the TPC of the samples differed significantly depending on the samples were between 55.73 mg GAE/100 g and 278.01 mg GAE/100 g. In the literature, the TPC values of the oil and seeds from which these wastes were obtained were mentioned since the phenolic content of the flaxseed oil by-products and black cumin seed oil by-products we used for this study was not studied in the literature. TPC of black cumin seed oil was determined as 114 mg GAE/100g oil [126] while the TPC value of flaxseed was given as 61.76-85.24 g GAE/g [127], 47.01 μ g GAE/g for methanolic extracts [128], and 349.70-511.60 mg/100g [129]. In this study, BOB had the highest amount of TPC, followed by FOB. The greater phenolic content in the by-product could be explained by the pretreatment procedure used before cold pressing. As a result of these approaches, the extractability of phenolic compounds from the plant matrix can be increased [130]. The TPC of the black cumin seed ranged from 281.8 to 292.5 mg/100g [131]. The TPCs of coconut meals were determined to be 77.5 mg GAE/ 100g [132]. In this study, The TFC values of COB were determined as 67.66 mg GAE/100g. As seen, our results showed consistent with the literature findings.

Different extraction methods and cultivars can produce different results in the literature. Phenolic compounds are bioactive molecules obtained from plants that have a high antioxidant impact and can be employed as natural antioxidants in an emulsion. They may be located near the oil-water interface, thereby preventing oxidation of oil/water emulsions. These results suggest that cold-pressed oil by-products, particularly BOB and FOB, are high in phenolic compounds and should be investigated for use in emulsions to prevent oxidation. Also, bioactive

compounds may provide possible health benefits such as anti-inflammatory, antiatherogenic, and antimutagenic.

3.2.2 The antioxidant capacity (CUPRAC)

The antioxidant capacity of the by-products was determined by CUPRAC. CUPRAC values ranged from 106.32 to 630.77 mg Trolox/100 g, and they significantly differed from each other. Table 3.3 showed that the TFC of the samples differed significantly depending on the by-product types, which were between 28.57 mg CE/100 g to 145.34 mg CE/100 g. BOB had the highest TFC values as well as TPC and CUPRAC values.

Table 3. 3 The bioactive properties of by-products

The bioactive properties	BOB	COB	FOB	POB
TPC (mg GAE/ 100 g)	278.01±8.14 ^a	67.66±4.38 ^c	190.99±6.58 ^b	55.73±0.31 ^c
CUPRAC (mg Trolox/ 100 g)	630.77±51.65 ^a	106.32±12.80 ^d	388.21±1.77 ^c	488.84±6.62 ^b
TFC (mg CE/100g)	145.34±4.35 ^c	28.57±0.17 ^c	89.31±2.01 ^b	33.92±0.18 ^c

¹TPC: total phenolic content, CUPRAC: the cupric reducing antioxidant capacity, TFC: total flavonoid content, BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods (p < 0.05).

3.2.3 The individual phenolic compounds

Table 3.4 indicated that individual phenolic compounds of BOB, COB, FOB, and POB and 15 phenolic compound standards were used to assess the individual phenolics of the by-products. As seen in Table 3.4, the number of individual phenolics varied significantly (P<0.05) depending on the types of by-products.

Just six of the standard phenolic compounds were found in COB, while all of them were found in BOB. Protocatechuic acid, *p*-Hydroxybenzoic acid, myricetin, and syringic acid were found to be higher in BOB. On the other hand, catechin and quercetin levels were found to be greater in POB and FOB, respectively.

Table 3. 4 Individual phenolic contents of by-products

Phenolic compounds	Amount of phenolics (mg/100 g)			
	BOB	COB	FOB	POB
Gallic acid	476.88±4.86 ^a	386.28±2.63 ^c	271.34±11.55 ^d	418.40±13.40 ^b
Protocatechuic acid	768.72±6.16 ^a	nd	172.37±15.81 ^b	213.06±9.23 ^b
Chlorogenic acid	280.14±6.96 ^b	nd	nd	2312.42±22.91 ^a
<i>p</i>-Hydroxybenzoic acid	864.04±9.92 ^a	0.09±0.00 ^c	0.16±0.01 ^b	0.37±0.01 ^b
Syringic acid	910.59±13.72 ^a	0.004±0.00 ^c	0.12±0.01 ^b	0.08±0.00 ^b
Ellagic acid	254.74±10.42 ^b	nd	227.86±8.62 ^c	298.48±11.60 ^a
<i>m</i>-Coumaric acid	0.34±0.02 ^b	nd	0.47±0.01 ^a	0.37±0.02 ^b
<i>o</i>-Coumaric acid	0.16±0.01 ^b	nd	163.67±5.88 ^a	0.25±0.01 ^b
Chrysin	0.002±0.00 ^b	nd	0.03±0.01 ^a	nd
Caffeic acid	200.64±11.05 ^a	174.24±6.53 ^a	129.51±4.59 ^b	139.78±7.69 ^b
<i>p</i>-Coumaric acid	0.53±0.00 ^b	0.08±0.01 ^d	0.60±0.01 ^a	0.13±0.00 ^c
Ferulic acid	0.78±0.03 ^b	0.45±0.01 ^c	225.25±5.83 ^a	0.34±0.01 ^c
Myricetin	870.26±14.32 ^a	nd	873.18±7.42 ^a	268.49±6.00 ^b
Quercetin	429.71±3.58 ^b	nd	2030.81±5.81 ^a	417.30±6.63 ^b
Kaempferol	160.17±8.22 ^a	nd	164.14±4.05 ^a	158.83±4.55 ^a

¹BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods ($p < 0.05$).

3.2.4 The in-vitro bio-accessibility of TPC

Table 3.5 indicated that the bioaccessibility (the release of compounds from plant matrices) of TPC of the cold-pressed oil by-products (BOB, COB, FOB, and POB) was examined by an in vitro model simulating gastrointestinal conditions. The cold-pressed oil extraction may cause a breakdown of the plant matrix and increased phenolic release. For the extracts of by-products (BOB, COB, FOB, and POB), TPC values were determined as 14.05 mg GAE/100 g, 6.78 mg GAE/100 g,

11.57 mg GAE/100 g, and 6.68 mg GAE/100 g in the IN fraction while 13.77 mg GAE/100 g, 4.82 mg GAE/100 g, 49.44 mg GAE/100 g, and 12.53 mg GAE/100 g in the OUT fraction, respectively. The percentage of recovery values were given as the ratios of the values obtained for the IN fraction to the values obtained for the initial (before digestion) values and then multiplied by 100 (Table 3.5). These recovery (%) values varied significantly depending on the type of by-products. The maximum and minimum TPC recovery were found in COB and FOB samples, respectively. The differences in in-vitro digestibility levels could be explained by the varied phenolic compound types and their connections with stomach and dietary ingredients [133]. When evaluating polyphenol digestion, dietary components such as proteins, carbohydrates (e.g. fiber), and fat, as well as their interactions with polyphenols, have rarely been considered. Protein in the dietary matrix has been attributed to a complex form with procyanidins with a high degree of polymerization, which may result in the reduced bioaccessibility of these phenolic compounds [134].

Table 3. 5 In-vitro bioaccessibility of TPC of by-products.

Digestion phase		BOB	COB	FOB	POB
TPC (mg GAE/100 g)	Initial	190.99±6.58 ^b	55.73±0.31 ^c	278.01±8.14 ^a	67.66±4.38 ^c
	Pg	47.57±0.38 ^b	9.75±0.25 ^d	58.38±2.32 ^a	12.85±0.63 ^c
	In	14.05±0.07 ^a	6.78±0.11 ^c	11.57±0.02 ^b	6.68±0.19 ^c
	Out	13.77±1.25 ^b	4.82±0.63 ^c	49.44±0.75 ^a	12.53±1.88 ^b
	Recovery (%)	7.35±0.03 ^b	12.16±0.20 ^a	4.16±0.01 ^c	9.87±0.27 ^b

¹TPC (mg GAE/100 g): total phenolic content, BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods (p < 0.05).

3.3 The antimicrobial activity of the cold-pressed oil by-products

Table 3.6 indicated that the extracts of by-products a showed antimicrobial activity against 4 common food pathogens, *S. aureus* ATCC 29213, *E. coli* ATCC

25922, *S. Typhimurium* ATCC 14028, and *L. monocytogenes* ATCC 13932. The inhibition zone diameters were determined from 8.50 mm to 14.83 mm for *S. aureus* ATCC 29213 while 6.83 mm to 12.50 mm for *E. coli* ATCC 25922, 8.33 mm to 13.50 mm for *S. Typhimurium* ATCC 14028, and 7.33 mm to 11.25 mm for *L. monocytogenes* ATCC 13932. As a result, all by-product extracts exhibited antibacterial activity against the tested bacteria.

Table 3. 6 Antimicrobial activity of by-products

M/OS	Inhibition zone diameter (mm)			
	BOB	COB	POB	FOB
<i>S. aureus</i> ATCC 29213	14.83±0.76 ^a	8.50±0.50 ^d	10.83±1.04 ^c	12.42±0.38 ^b
<i>E. coli</i> ATCC 25922	12.50±0.50 ^a	6.83±0.29 ^d	8.67±0.58 ^c	10.50±0.50 ^b
<i>S. typhimurium</i> ATCC 14028	13.50±0.50 ^a	8.33±0.58 ^b	9.25±0.66 ^b	13.17±0.29 ^a
<i>L. monocytogenes</i> ATCC 13932	10.33±0.29 ^b	7.58±0.38 ^c	7.33±0.29 ^c	11.25±0.25 ^a

¹BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods ($p < 0.05$).

Gram-positive bacteria are more resistant to all extracts than Gram-negative bacteria. Among all the pathogens tested, *S. aureus* ATCC 29213 was found to be the most sensitive strain. BOB, on the other hand, was found to be more effective against test pathogens than FOB, POB, and COB. The inhibition diameters and TPC value had a positive correlation ($p > 0.90$). In conclusion, the results showed that all extracts have an antimicrobial effect on common food pathogens *S. aureus* ATCC 29213, *S. Typhimurium* ATCC 14028, *E. coli* ATCC 25922, and *L. monocytogenes* ATCC 13932.

Pag, Radu, Draganescu, Popa and Sirghie [135] investigated that the crude and hydrolyzed extracts of cold-pressed flaxseed oil by-product showed antibacterial activity against *S. aureus*, *P. aeruginosa*, and *E. coli* due to phenolic compounds, including lignans. It can be said that the synthesization of lignan-rich polyphenolic extracts from flaxseed cake is a sustainable strategy because of the great

recoverability of this cold-pressed oil industry by-product. Also, this by-product may be used with synthetic food additives because of the source of antioxidants and antibacterial extracts.

3.4 The determination of optimum by-product contents for the effects on rheological properties of the low-fat salad dressing

In this part, the rheological properties (flow behavior, dynamics, and 3-ITT) of low-fat salad dressing samples obtained from the by-products (BOB, COB, FOB, and POB) were analyzed to determine the possible use of these by-products as fat substitutes in low-fat salad dressing. Therefore, salad dressings containing 1-5% by-products were prepared and these salad dressing samples were compared with low-fat and high-fat salad dressing samples in terms of rheological properties.

3.4.1 The steady shear rheological properties

Flow behavior rheological properties of salad dressings are an important indicator in determining the quality of these products. Figure 3.1 demonstrates the flow behavior of the low-fat and high-fat control salad dressing samples and the low-fat salad dressing contained with by-products (BOB, COB, FOB, and POB). As seen in Figure 3.1, all salad dressing samples exhibited shear-thinning flow behavior. In other words, the apparent viscosity values of the salad dressing samples decrease when the shear rate increases. The viscosity values of the salad dressings decreased due to the increasing shear rate. This can be explained by the weakening of the weak bonds between the molecules in the product and the weakening of the interaction between the components as a result of the applied force. In the literature, previous studies have also reported that emulsions with different oil concentrations showed shear-thinning flow behavior [40, 136]. This behavior was explained by the severe shear-induced structural collapse connected to an oil droplet deflocculating process [137].

In comparison to the other samples, C2 samples (10% oil) had low viscosity (Figure 3.1-A). The oil concentration of O/W emulsions has a considerable influence on sample flow characteristics, as shown in Figure 3.1-A. When FOB-3 (enriched with 3% FOB) was compared to control samples (C1 and C2), the rheological properties of FOB-3 were close to the rheological properties of C1

(30% oil). In other words, 3% addition of FOB was sufficient to regain the weakened structure as a result of the 20% reduction in oil. The FOB-5 samples showed more consistency compared to the high-oil control samples.

Figure 3.1-B represents the flow behavior properties of the control samples (C1 and C2) and POB enhanced samples (POB-1, POB-3, and POB-5). POB-1 showed a similar trend with C2, indicating that the addition of 1% FOB was insufficient in the development of a more consistent character, which is weakened by the decrease in the oil ratio. The steady-state rheological properties of the BOB and COB enriched samples were shown in Figures

3.1-C and 3.1-D, respectively. As shown, BOB and COB had a very strong consistency character, and a 1% addition of BOB and COB displayed a similar flow behavior trend as C2 samples. As seen in Figures 3.1-C and 3.1-D, the salad dressing samples have shear stress values at the initial shear rate, that is, they showed the presence of yield stress. This flow behavior is the Herschel Bulkley flow behavior, which includes the yield stress expected from salad dressings.

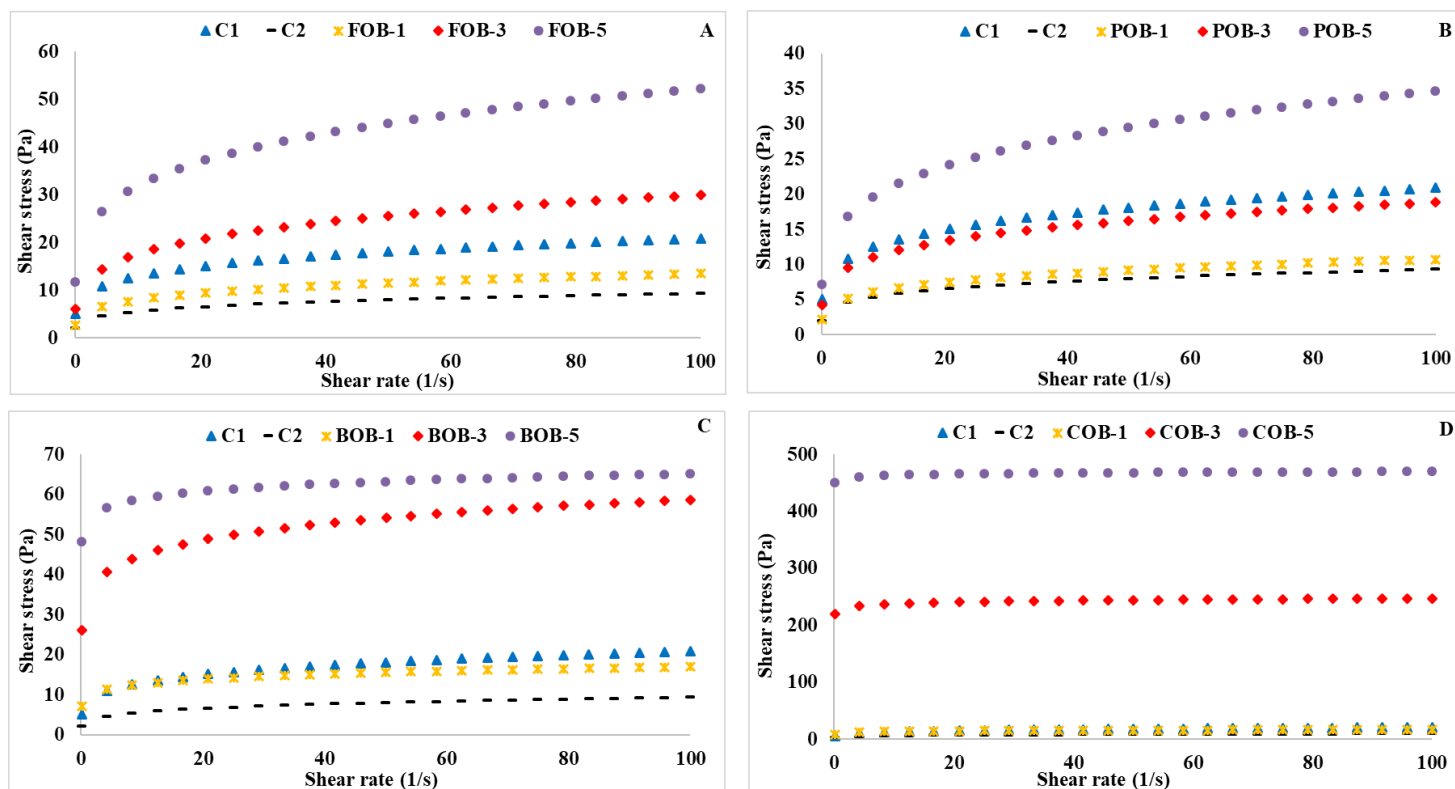


Figure 3. 1 Flow curve of salad dressing samples ⁴

⁴ C1: control sample with 30% oil, C2: control sample with 10% oil, BOB: cold-pressed black cumin oil by-product; COB: cold-pressed coconut oil by-product; FOB: cold-pressed flaxseed oil by-product; POB: cold-pressed pumpkin seed oil by-product; BOB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % BOB; COB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % COB; FOB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % FOB; POB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % POB

The Power-law parameters calculated by using steady-state rheological data were seen in Table 3.7. Modeling shear stress versus shear stress values utilizing the Power-Law model yielded K and n values. The Power-Law model could be used to model flow behavior and rheological properties effectively ($R^2 > 0.95$). All samples had a value less than 1, meaning that they all had a shear-thinning character. The samples' K values were statistically different ($P < 0.05$). The K value of the C1 and C2 samples were 8.05 and 3.61 Pa sⁿ.

Table 3. 7 The power law parameters of the salad dressing samples

Samples	K (Pa s ⁿ)	n	R ²	K' (Pa s ⁿ)	n'	R ²	K'' (Pa s ⁿ)	n''	R ²
C1	8.05 ^b	0.21	0.99	13.68 ^c	0.25	1.00	5.35 ^b	0.17	0.89
C2	3.61 ^c	0.22	0.99	1.51 ^e	0.74	0.99	1.20 ^d	0.37	0.82
FOB-1	4.71 ^c	0.23	0.99	2.97 ^d	0.58	0.97	2.71 ^d	0.25	0.97
FOB-3	10.29 ^b	0.23	0.99	14.88 ^b	0.28	0.99	7.93 ^b	0.18	0.83
FOB-5	19.31 ^a	0.22	0.99	40.84 ^a	0.22	0.99	12.62 ^a	0.22	0.94
C1	8.05 ^b	0.21	0.99	13.68 ^c	0.25	1.00	5.35 ^c	0.17	0.89
C2	3.61 ^c	0.22	0.99	1.51 ^e	0.74	0.99	1.20 ^e	0.37	0.82
POB-1	3.75 ^c	0.23	0.99	3.08 ^d	0.55	0.99	2.48 ^d	0.27	0.97
POB-3	8.05 ^b	0.19	0.95	16.94 ^b	0.14	0.88	30.11 ^b	0.09	0.83
POB-5	12.00 ^a	0.23	0.99	68.55 ^a	0.18	0.97	127.06 ^a	0.08	0.92
C1	8.05 ^c	0.21	0.99	13.68 ^c	0.25	1.00	5.35 ^d	0.17	0.89
C2	3.61 ^d	0.22	0.99	1.51 ^d	0.74	0.99	1.20 ^e	0.37	0.82
BOB-1	10.38 ^c	0.12	0.95	14.43 ^c	0.15	0.98	15.91 ^c	0.12	0.81
BOB-3	34.19 ^b	0.12	0.91	35.63 ^b	0.10	1.00	239.91 ^b	0.05	0.90
BOB-5	53.17 ^a	0.13	0.99	194.92 ^a	0.10	1.00	229.98 ^a	0.07	0.86
C1	8.05 ^c	0.21	0.99	13.68 ^d	0.25	1.00	5.35 ^d	0.17	0.89
C2	3.61 ^d	0.22	0.99	1.51 ^e	0.74	0.99	1.20 ^e	0.37	0.82
COB-1	10.86 ^c	0.09	0.99	18.31 ^c	0.07	0.99	236.08 ^c	0.06	0.83
COB-3	228.22 ^b	0.02	0.99	72.76 ^b	0.09	1.00	811.38 ^b	0.06	0.83
COB-5	456.26 ^a	0.01	0.99	196.81 ^a	0.08	0.97	1894.50 ^a	0.07	0.91

¹C1: control salad dressing with 30% oil; C2: control salad dressing with 10% oil; BOB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % BOB; COB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % COB; FOB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % FOB; POB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % POB.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods ($p < 0.05$).

The K value of all of the salad dressing samples enriched with by-products was higher than the C1 and C2 samples, except for FOB-1 and POB-1. The K value of FOB1, FOB2, and FOB 3 was 4.71, 10.29, and 19.31 Pa sⁿ. The K value of the POB1, POB3, and POB5 were found as 3.75, 8.05, and 12.00 Pa sⁿ. As can be seen, FOB-1 and POB showed similar K values to the samples C2. The K value of the samples BOB1-5 was found to be 10.38, 34.19, and 53.17 Pa sⁿ, respectively, and was found as 10.86, 228.22, and 456.26 Pa sⁿ for COB1-5, respectively, indicating that FOB-3, POB-3, BOB-1, and COB-1 exhibited similar K values to the C1 samples ($P < 0.05$). In total, 3% and 5% of the BOB-and COB-enriched samples exhibited high K values compared to C1. This result can be associated with the polysaccharides with water-holding properties in BOB and COB [138]. Previous research has found that COB and BOB have high levels of fiber and protein [27, 139]. Because of the high fiber and protein content in the COB, the water remained in the continuous phase, and the decreased viscosity and consistency were recovered with the decrease of the oil. These findings indicated that cold-pressed oil by-products may be utilized to improve the structure of low-fat salad dressing samples.

3.4.2 The dynamic rheological properties

Figure 3.2 exhibited the salad dressing samples' dynamic rheological properties. Except for C2, all samples exhibited viscoelastic solid behavior because G' was higher than G'' along with all frequency values (Figure 3.2). These findings may be attributed to a less compressed structure with lower oil content [140]. Except for FOB-1 and POB-1, all by-product enriched samples had greater G' and G'' values than control samples (C1 and C2), meaning that the addition of by-products improved the less compact structure as a result of reducing the oil content. The samples enriched with more than 3% BOB and COB revealed a very strong solid-like structure. A very compact structure is not desired in order not to adversely affect the flow of salad dressings during consumption. The Power-law model was utilized to represent G' and G'' values obtained for angular velocity, as well as K' , K'' , n' , and n'' values. Except for C2, K' value was greater than K'' value in all salad dressing samples. This study provided more evidence of the viscoelastic solid nature of salad dressing samples. FOB-1 and POB-1 had lower K' values than

C2 samples, but FOB-3, POB-3, BOB-1, and COB-1 had comparable K' values. Finally, the enrichment of the by-product enhanced the solid-like structure of low-fat salad dressing and provided it a desired viscoelastic nature, as shown in Figure 3.2.

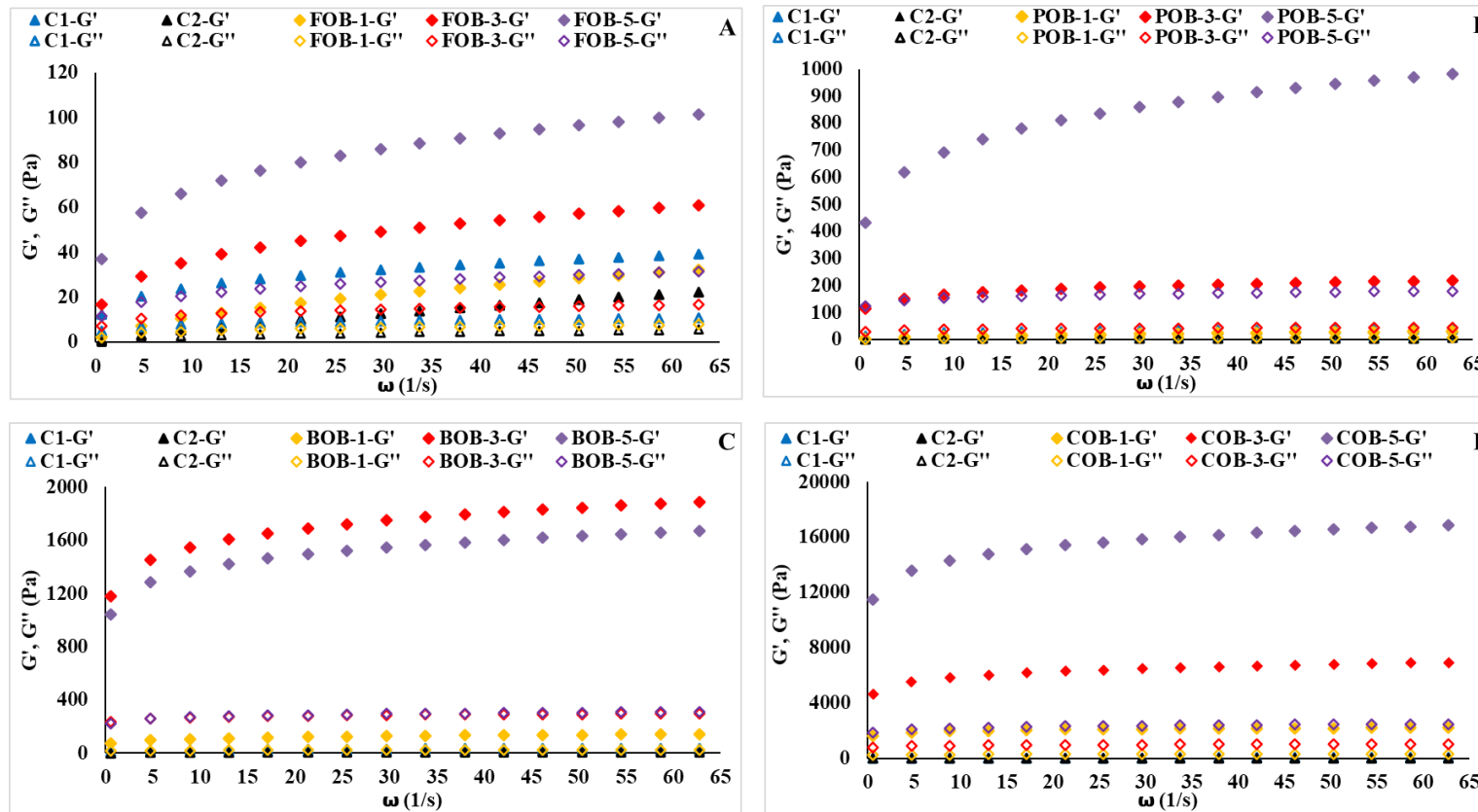


Figure 3. 2 The dynamic rheological properties of salad dressings⁵

⁵ G' (Pa): the storage modulus; G'' (Pa): the loss modulus; C1: control salad dressing with 30% oil; C2: control salad dressing with 10% oil; BOB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % BOB; COB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % COB; FOB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % FOB; POB-1, 3 & 5: low fat salad dressing contained 1, 3, & 5 % POB

3.5 The rheological properties of the salad dressings contained cold-pressed flaxseed oil by-product

In this part of the study, the formulation optimization of salad dressings containing FOB was conducted to determine the optimum amount of these by-products. For this purpose, 17 different formulations were prepared with 0.5-1.5% FOB. The optimum FOB amount was determined by RSM and CCD.

3.5.1 The steady shear rheological properties

Figure 3.3 indicated the shear stress–shear rate relationships of salad dressing samples contained FOB (obtained from 17 different experimental points). The shear-thinning flow behavior type is a desired flow behavior feature in low-fat O/W emulsions [141]. The samples displayed a downward curve on the shear rate axis, indicating that all samples have pseudoplastic features, which are desired rheological qualities for salad dressing, including low-fat O/W emulsions, as shown in Figure 3.3. In other words, the intermolecular interaction between highly branched polysaccharides, proteins, and water break down, causing viscosity to decrease as the shear rate increases [142]. Previous research has also found this type of flow behavior in O/W emulsions with low-fat content (for example, salad dressings) [28, 143]. Pero, Emam-Djomeh, Yarmand and Samavati [144] investigated that the rheological properties of low-fat salad dressing samples contained salep as a stabilizer and these samples showed the shear thinning behavior.

According to Figure 3.3, even though sample 2's oil content was half that of sample 1, a greater viscosity value was achieved with the addition of 1% FOB. Although the oil content of samples influences the flow characteristics, that are viscosity and consistency, the rheological properties of low concentrations (1%) of FOB enhanced samples exhibited similar rheological properties to those of high-fat content samples. Sun, Liu, Feng, Xu and Zhou [11] reported that the viscosity of emulsions increased with the addition of flaxseed gum (0.1-0.5%).

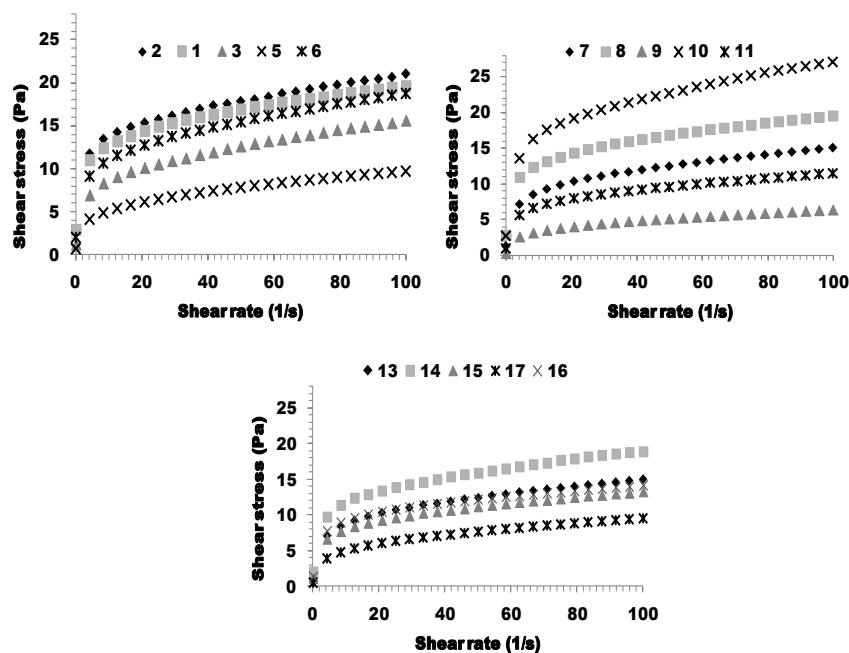


Figure 3. 3 Steady shear rheological properties of salad dressing contained FOB⁶

The rheological parameter of the salad dressing samples was discovered by fitting the Power Law model to the experimental data and measuring the K and n values of the 17 different runs, as shown in Table 3.8. The Power Law model is commonly used to identify shear-thinning fluids and may also be used to predict salad dressing flow behavior. According to Table 3.8, the R^2 value in the range of 0.98–1.00 showed that the Power-Law model can successfully model the flow behavior rheological properties of the salad dressing. The K and n values of the salad dressing were determined as 1.68–9.13 Pa s ^{n} and 0.21–0.29, respectively. As a consequence, the n values of all samples were less than 1, meaning that the salad dressings showed pseudoplastic flow behavior. Also, as the K value increased, the pseudoplastic character n value decreased.

In a study, salad dressings prepared with green lentils, chickpeas, and yellow peas were optimized according to the response surface method [145]. Flow behavior rheological properties were investigated and n value was found in the range of 0.16-0.49.

⁶ FOB: cold-pressed flaxseed oil by-product

Table 3. 8 Power-law parameters and zeta-potential values of the salad dressing contained FOB

Sample	XG (%)	FOB (%)	EYP (%)	Oil (%)	K(Pa s ⁿ)	n	R ²	ζ-potential
1	0.4	0.5	3.00	20.0	7.52±0.11	0.21±0.00	0.98	-32.7±0.86
2	0.4	1.5	3.00	10.0	7.88±0.00	0.21±0.00	0.99	-34.7±0.52
3	0.2	1.5	3.00	20.0	4.21±0.17	0.28±0.00	1.00	-34.8±0.31
4	0.3	1.0	3.00	15.0	3.86±0.09	0.25±0.00	0.99	-43.4±0.29
5	0.2	0.5	3.00	20.0	2.47±0.01	0.29±0.00	1.00	-50.0±0.94
6	0.3	1.0	3.00	20.0	6.12±0.26	0.25±0.00	1.00	-34.8±0.27
7	0.3	1.0	3.00	15.0	4.83±0.08	0.25±0.00	0.99	-33.2±0.31
8	0.2	1.5	3.00	10.0	2.67±0.12	0.27±0.00	0.99	-35.8±0.14
9	0.2	0.5	3.00	10.0	1.68±0.04	0.29±0.00	1.00	-45.8±0.11
10	0.4	1.5	3.00	20.0	9.13±0.25	0.23±0.00	0.99	-29.8±0.71
11	0.3	1.0	3.00	10.0	3.67±0.11	0.25±0.00	0.99	-45.5±1.03
12	0.3	1.0	3.00	15.0	4.66±0.01	0.25±0.00	0.99	-39.2±1.05
13	0.3	1.5	3.00	15.0	5.78±0.14	0.24±0.00	0.99	-36.4±0.41
14	0.4	1.0	3.00	15.0	6.63±0.06	0.23±0.00	0.99	-40.5±0.61
15	0.3	0.5	3.00	15.0	4.49±0.01	0.23±0.00	0.99	-43.1±0.73
16	0.4	0.5	3.00	10.0	5.50±0.06	0.21±0.00	0.99	-44.6±0.80
17	0.2	1.0	3.00	15.0	2.62±0.16	0.29±0.00	1.00	-35.8±1.12

¹EYP: egg yolk powder; FOB: cold-pressed flaxseed oil by-product; XG: xanthan gum; K (Pa sⁿ): consistency coefficient, n: the flow behavior index values; ζ-potential: zeta potential.

In another study, the rheological properties, emulsion stability, and microstructural properties of salad dressings produced using oil, egg yolk, salt and salep were investigated [144]. When the flow behavior c

haracteristics of salad dressings produced with different formulations were examined, it was revealed that the viscosity decreased (shear thinning) in response to increasing shear rate.

In a study, the rheological properties of salad dressings produced using chia seed oil by-product were investigated by [6]. The R^2 value range being between 0.95-0.99 shows the suitability of the Power Law model in determining the flow behavior of salad dressings. K and n values differed according to the formulation content and were found as 0.5521-15.8747 Pa sⁿ, 0.1266-0.3818, respectively.

According to Table 3.8, the samples that contained higher oil, gum, and FOB content had a higher K value. When the oil content was decreased, a dramatic

decrease in the pseudoplastic behavior of the salad dressings occurred, namely a decrease in the K value. The addition of FOB resulted in salad dressings with a comparable K value as those with high oil content. According to Table 3.8, while sample-2 (10% oil and 1.5% FOB) had a K value of 7.88 Pa sⁿ, sample-1 (20% oil and 0.5% FOB) had a K value of 7.52 Pa sⁿ, despite both samples containing the same XG (0.4%) quality. This was achieved by an increase in the concentration of FOB (only %1). By adding FOB to salad dressing sample with low oil content, K value increased and n value decreased, meaning that the pseudoplastic character. The strong water binding ability of flaxseed polysaccharides (a neutral arabinoxylan (75%) and an acidic rhamnogalacturonan (25%) in the seed coat might explain that K value increased by increasing FOB content [146].

3.5.2 The ζ -potential value and emulsion stability index

The ζ -potential value is a significant factor for deciding the stability of salad dressings. Table 3.8 showed that the salad dressing samples' ζ -potential values ranged from -29.8 mV to -50.0 mV, indicating that the proteins around the oil droplets became negatively charged. Zhao, Long, Kong, Liu, Sun-Waterhouse and Zhao [147] also found that the product shifts towards the negative charge in the ζ -potential (until -28.05 mV) as the gum ratio and FOB increase. According to our findings, negatively charged proteins and polysaccharides of FOB bound to oil droplets escalated the ζ -potential of emulsions. Salad dressing samples with the highest zeta potential were found to have a value of -50.0 mV. Salad dressing samples with high zeta potential values may have been expected to have long-term stability [143]. A visual approach was also used to measure the emulsion consistency of the 17 different samples. After 28 days of the storage period, no phase separation was observed for the 17 samples (Figure 3.4). The findings of ζ -potential revealed that FOB can be utilized as stabilizers for the increase of the low-fat salad dressing stability.

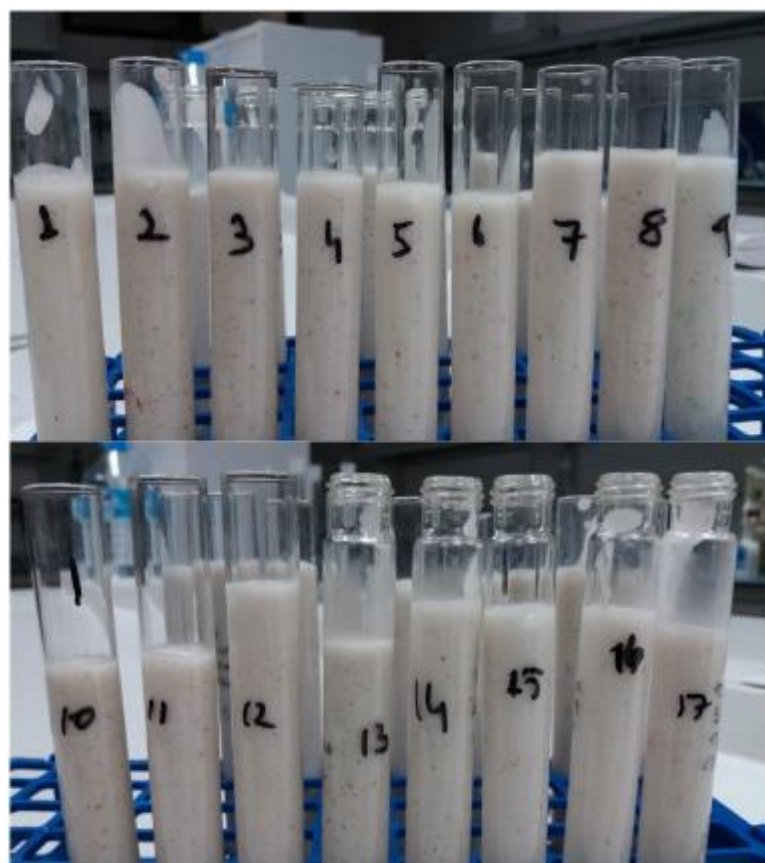


Figure 3. 4 Salad dressing samples contained FOB⁷ obtained after 28 days storage periods

3.5.3 The quadratic model parameters of K, K' and K''

The influence of the formulation on the K, K', and K'' values of the salad dressing samples obtained using the quadratic model and the response surface methodology (RSM) was shown in Table 3.9.

The quadratic model successfully characterized the influence of the formulations on the K, K', and K'' values, as evidenced by the model's R^2 value (between 0.96 and 0.97). The components had a substantial influence on the K value ($p < 0.05$), while the lack of fit was insignificant ($p > 0.01$). XG, FOB, and oil of formulation were significant for the model, as demonstrated by the quadratic model.

⁷ FOB: cold-pressed flaxseed oil by-product

Table 3. 9 The significance of the regression models generated (F and p-value) as a consequence of RSM

<i>Source</i>	<i>df</i>	<i>K</i>			<i>K'</i>			<i>K''</i>		
		Mean square	F-Value	p-value (Prob > F)*	Mean square	F-Value	p-value (Prob > F)*	Mean square	F-Value	p-value (Prob > F)*
<i>Model</i>	9	7.48	31.19	<0.0001	22.34	21.27	0.0003	2.58	24.47	0.0002
<i>Linear</i>										
<i>A-XG</i>	1	52.93	220.74	<0.0001	132.50	126.18	< 0.0001	16.54	156.78	< 0.0001
<i>B-FOB</i>	1	6.43	26.80	0.0013	16.63	15.83	0.0053	1.73	16.38	0.0049
<i>C-Oil</i>	1	6.49	27.05	0.0013	41.38	39.41	0.0004	4.31	40.83	0.0004
<i>Cross Product</i>										
<i>AB</i>	1	0.20	0.82	0.3943	2.52	2.40	0.1654	0.019	0.18	0.6853
<i>AC</i>	1	0.11	0.46	0.5180	1.07	1.02	0.3462	0.11	1.06	0.3370
<i>BC</i>	1	1.10×10 ⁻⁴	4.59×10 ⁻⁴	0.9835	0.48	0.45	0.5224	0.047	0.45	0.5241
<i>Quadratic</i>										
<i>A²</i>	1	0.013	0.056	0.8201	0.22	0.21	0.6604	0.023	0.22	0.6520
<i>B²</i>	1	0.52	2.16	0.1848	0.47	0.44	0.5262	0.073	0.69	0.4339
<i>C²</i>	1	0.11	0.44	0.5278	1.66	1.58	0.2493	0.20	1.92	0.2086
<i>Lack of Fit</i>	5	0.23	0.84	0.6227	0.17	0.052	0.9956	0.039	0.14	0.9637
<i>R-Squared</i>		0.9757			0.9647			0.9692		
<i>Adj R-Squared</i>		0.9444			0.9194			0.9296		

¹FOB: cold-pressed flaxseed oil by-product; XG: xanthan gum; K, K', and K'' (Pa sⁿ): consistency coefficient values.

The impact of each component and their interaction on the K value of the salad dressings were demonstrated in Figure 3.5. As seen in Figure 3.5, the K value increased as the concentrations of oil, XG, and FOB increased throughout the range.

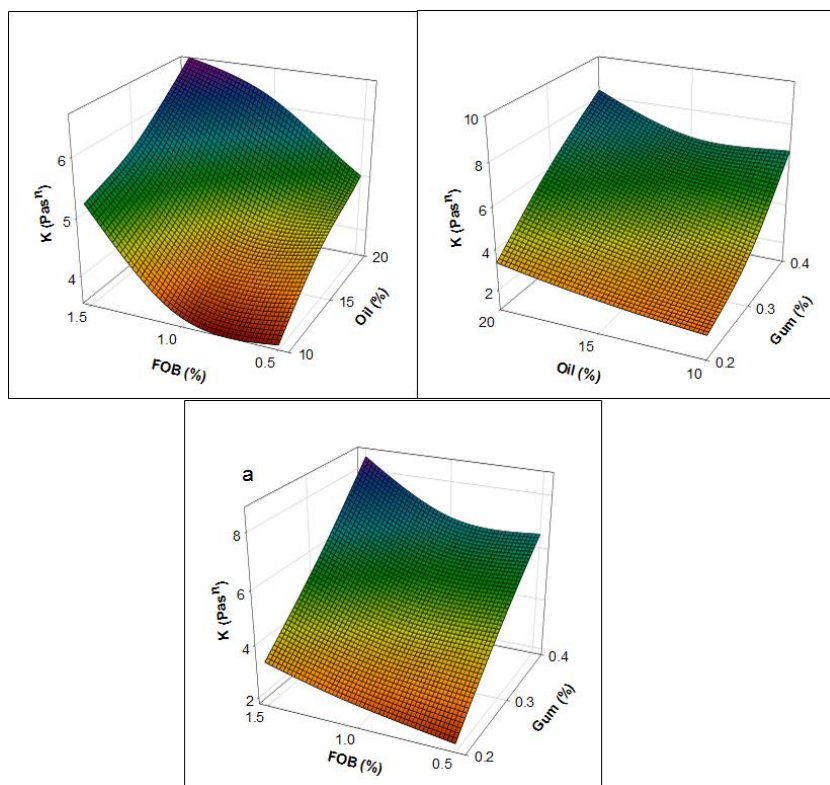


Figure 3. 5 The impact of the different formulations of parameters on the K value of salad dressing contained FOB⁸

FOB followed the same pattern as oil and XG content, implying that using FOB instead of oil and XG resulted in identical rheological characteristics. The K value increased considerably, and the oil and emulsifier had a synergistic effect by raising the XG content, according to Sikora et al. (2008), the K value significantly increased, and the oil and emulsifier had a synergistic impact by increasing the XG content. Other studies showed a rise in the usage of stabilizers, oil, and cold-pressed by-products, as well as an increase in the K value of salad dressing and related products [32, 40, 143].

⁸ K (Pa sⁿ): consistency coefficient, FOB: cold-pressed flaxseed oil by-product

Optimization was carried out according to RSM in salad dressings prepared by using green lentil, chickpea, and yellow pea protein isolates by Ma, Boye and Simpson [38]. The effect of formulation components on the K value was investigated and the K value increased due to the synergistic interaction between green lentils and egg yolk. This is because the solid particles in the vinaigrette emulsions increase the resistance to flow, thus improving the viscous properties of the vinaigrettes, and its viscosity is expected to increase as the levels of egg yolk and green lentil protein isolate increase. In addition, as the emulsifier (egg yolk and green lentil protein) content increases, the main reason for the observed gain trend in K values can be explained by the development of a compact network structure between molecules. On the other hand, as the oil content decreases, a less compact network structure can be formed with the increase in the mean distance between the droplets, which reduces the K value. This finding is consistent with the results obtained in previous studies [39, 71], which indicated that viscous properties increased as the oil concentration increased in water-in-oil emulsion and mayonnaise samples.

These studies showed compatibility with our results in terms of both the K value and the n value. Based on the properties stated in the researches, cold-pressed oil by-products (BOB, COB, FOB, and POB) shows a stabilizer feature due to the polysaccharide content, and it is understood that they can be used as an emulsifier by adsorbing the protein content in the interface region and showing surface-active properties. Based on these properties, cold-pressed oil by-products increased the consistency coefficient value (K value). These by-products can be used in low-fat salad dressing formulations as emulsifiers and stabilizers. Also, they are adsorbed at the interface due to the protein content, reducing the interfacial tension, which shows the surface-active property, and causing an increase in the K value. Cold-pressed oil by-products (BOB, COB, FOB, and POB) significantly increased the K value of the salad dressing by affecting the viscosity due to the solid particle content. The oil, emulsifier, stabilizer, and cold-pressed oil by-products (BOB, COB, FOB, and POB) cause a significant increase in the consistency coefficient (K value), explaining that the synergistic interaction

between the ingredients. When compared with the studies obtained, our results were following the literature findings.

3.5.4 The dynamic rheological properties

The dynamic rheological characteristics of the salad dressings were depicted in Figure 3.6. For all samples, G' and G'' values increased with increasing angular frequency and the G' values were higher than G'' values in all frequency ranges. This indicates that all samples had a solid-like property, as is the typical property of salad dressing samples. As seen in Figure 3.6, the formulation was impacted by G' value and the increase of each component (FOB, XG, and oil) caused a higher G' value, showing that FOB enhanced the desired solid-like behavior of the salad dressings.

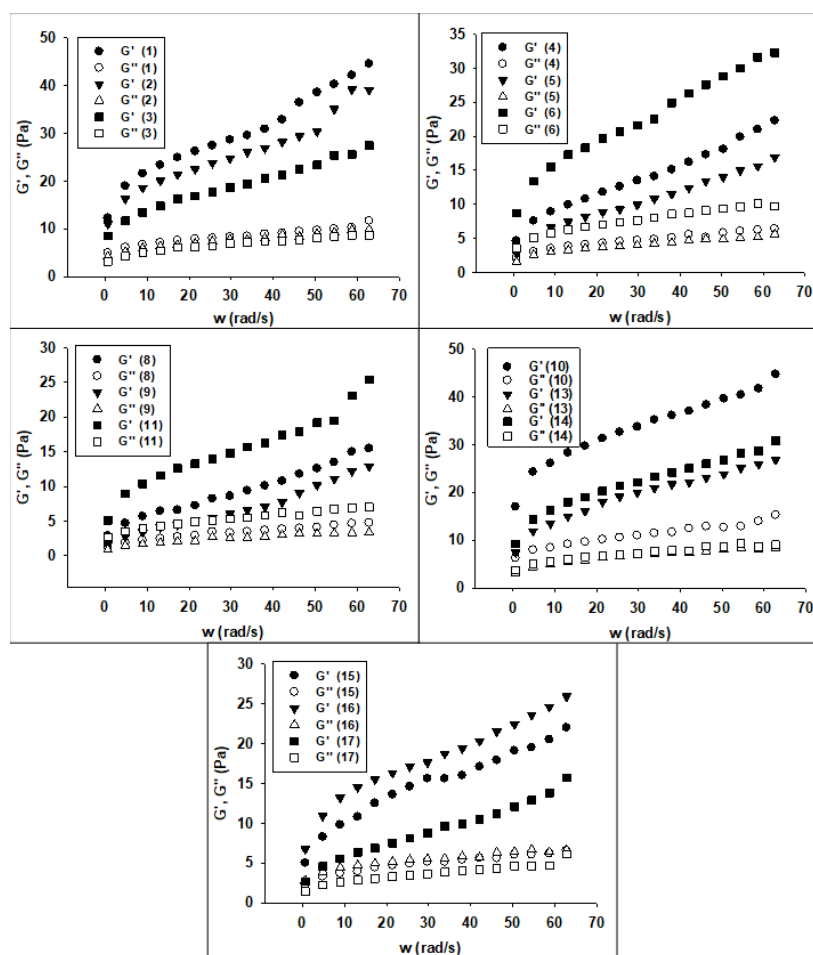


Figure 3. 6 The dynamic rheological properties of the salad dressing contained FOB⁹

⁹ G' (Pa): the storage modulus; G'' (Pa): the loss modulus; FOB: cold-pressed flaxseed oil by-product

Table 3.10 demonstrated that the Power-law model was adjusted to the data acquired from frequency rheological analysis, and K' , K'' , n' , and n'' , as well as R^2 , were calculated. As shown in Table 3.10, all K' values of all samples were greater than all K'' values (except FOB-9), indicating that the samples prepared using FOB were more primarily elastic, i.e., solid-like rather than viscous.

The main reason for the increase in the K' value with the increase in the amount of FOB is due to the polysaccharide structure of the FOB with high water retention. Polysaccharides are significant due to their colloidal, viscous, and gelling qualities. They enhance the viscoelastic solid character structure due to their function in the formation of dispersed structures with highly viscous characteristics and their high presence in FOB. This also happens at lower emulsifiers and oil levels. Due to the bond formation between molecules, especially the bonds formed between protein-polysaccharide structures, an increase in the G' , that is, the storage module occurs. However, the amount and type of emulsifier used are also important because of the bond formation between the protein-polysaccharide structure. Furthermore, because proteins have emulsifying capabilities in both egg yolk powder and FOB, they participate in chemical and physical reactions at the oil-water interface and play a key role in emulsion stability. Furthermore, as the amount of oil increases, the mobility of the continuous phase reduces, and a more viscous and stable structure forms. This is how the basic synergistic action of salad dressing components occurs. It can be said that the reason why the stabilizer substances among the components are significantly effective in both flow behavior and dynamic rheological properties compared to the others, both reduce the mobility of the continuous phase and cause a stable emulsion structure by forming a weak gel structure. As the oil content increases, the K' values increase because the continuous phase decreases and its mobility can be controlled with the increase of the oil content. However, with the use of FOB, the K' value increases, that is, by reducing the oil content and increasing the amount of FOB, low-fat salad dressing with the same consistency can be prepared.

In a study, the rheological, microstructural, sensory, and stability properties of salad dressings prepared by adding unprocessed and heat-treated lentil flour were investigated by Ma, Boye, Fortin, Simpson and Prasher [28]. It was stated that G'

values of all prepared salad dressings were higher than G'' values, and it was also stated that heat-treated lentil flours had higher G' values compared to control and unprocessed lentil flours.

Table 3. 10 Dynamic rheological properties of the salad dressing contained FOB

Run	$G' = K'(\omega)^{n'}$			$G'' = K''(\omega)^{n''}$		
	K'	n'	R ²	K''	n''	R ²
1	10.53±3.26	0.33±0.10	0.95	4.49±0.16	0.20±0.01	0.97
2	9.12±1.37	0.32±0.05	0.96	3.84±0.17	0.21±0.01	0.98
3	5.29±1.90	0.37±0.07	0.98	2.58±0.45	0.28±0.02	0.99
4	2.97±0.04	0.41±0.08	0.98	1.93±0.04	0.28±0.00	0.98
5	3.53±0.83	0.36±0.02	0.98	1.48±0.06	0.30±0.01	0.99
6	7.26±0.66	0.34±0.03	0.98	3.29±0.04	0.26±0.00	0.99
7	5.78±0.93	0.34±0.04	0.98	2.72±0.02	0.24±0.01	0.98
8	1.34±0.19	0.58±0.04	0.98	1.11±0.09	0.34±0.03	0.98
9	0.53±0.12	0.75±0.06	0.98	0.77±0.06	0.36±0.02	0.99
10	14.56±0.41	0.27±0.01	0.92	5.34±0.11	0.22±0.00	0.96
11	3.74±0.26	0.40±0.02	0.96	2.05±0.33	0.27±0.02	0.97
12	6.33±1.90	0.35±0.02	0.99	2.91±0.55	0.26±0.00	0.98
13	6.76±0.05	0.32±0.01	0.98	3.00±0.02	0.25±0.00	0.99
14	8.39±0.64	0.30±0.02	0.99	3.44±0.01	0.24±0.00	0.98
15	3.50±1.16	0.45±0.11	0.99	2.12±0.12	0.27±0.03	0.98
16	6.09±0.08	0.33±0.00	0.99	2.85±0.02	0.20±0.00	0.98
17	1.61±0.01	0.54±0.03	0.98	1.16±0.01	0.36±0.00	0.97

¹FOB: cold-pressed flaxseed oil by-product, G' (Pa): the storage modulus, G'' (Pa): the loss modulus, K' , K'' (Pa sⁿ): consistency coefficient values, n' , n'' : the flow behavior index values

Tadros [140] reported that the G' and G'' values of the salad dressings increased when the oil content of emulsions like salad dressing increased. Oil content has a significant influence on the rheological properties of salad dressings. Because of the lower oil content, the solid-like structure is reduced, resulting in a less compact structure. To improve emulsion stability and product quality, the solid-like structure of emulsions with decreasing oil content should be maintained. Although the oil content dropped (to 10%) in our study, the K' and G' values and viscoelastic characteristics rose with the increase of FOB content (by increasing 1%) because

of FOB's high water retention capacity. According to the results of this study, FOB might be utilized as a fat replacement in the preparation of low-fat O/W emulsion.

3.5.5 3-ITT rheological properties

O/W emulsions may be subjected to deformations during the production of emulsions (high-speed mixing and homogenization) and consumption of emulsions (shaking or squeezing the packed food). Permanent deformations are undesired, particularly in salad dressings with low oil content. As a consequence, the materials can regain their viscoelastic characteristics at rest after a specific duration of deformation. The 3-ITT is a rheological test that replicates the rheological behavior of salad dressing as they are deformed in LVR and non-LVR. The thixotropic behavior of the salad dressing was depicted in Figure 3.7. In the third period of Figure 3.7, all of the samples exhibited thixotropic behavior. After severe shear deformation, samples lost their viscoelastic character and recovered their viscoelastic character in a second interval. These results demonstrated that all emulsions should retain their viscoelastic characteristics throughout food processing that involves a substantial degree of abrupt deformation, such as pumping or homogenization, as well as consumption during pressing and shaking. For salad dressing type emulsions, this flow behavior is a desirable characteristic. According to Toker, Karasu, Yilmaz and Karaman [75], commercial mayonnaise samples exhibited thixotropic behavior. Akcicek and Karasu [143] reported the recoverable properties of samples enriched with chia seed oil by-products in the 3rd interval. These results are similar to those found in our research. According to the current study, a by-product of cold-pressed flaxseed oil improved the thixotropic behavior of salad dressing during rapid deformation.

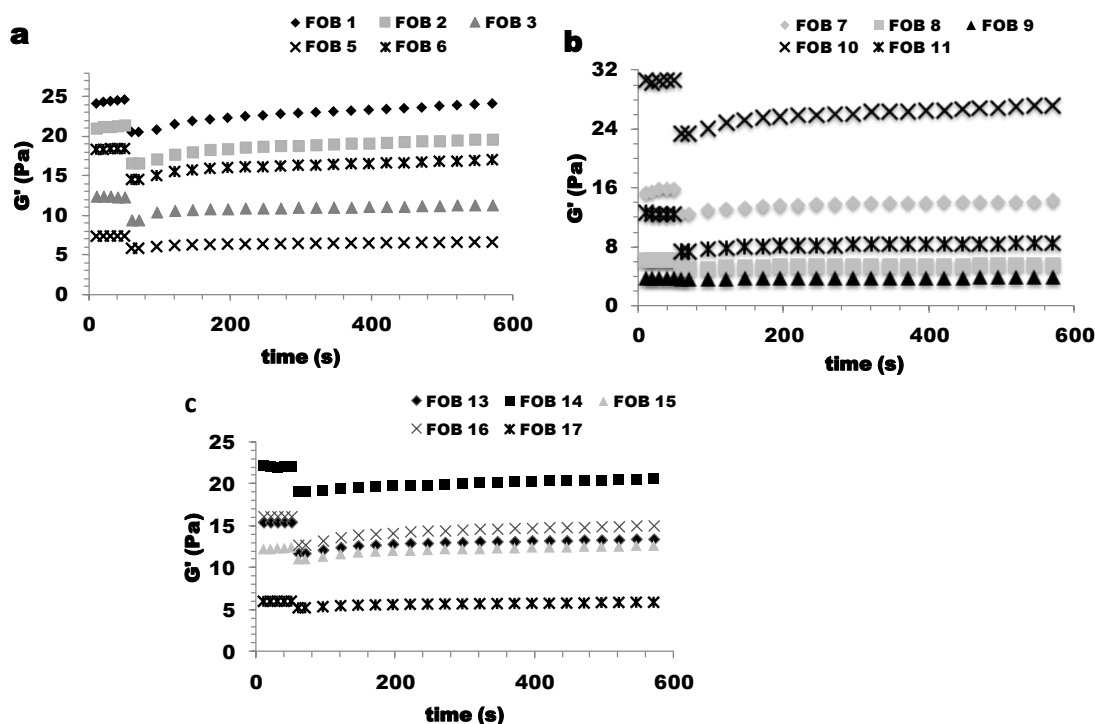


Figure 3. 7 Thixotropic behavior of the salad dressings contained FOB¹⁰

A second-order structural kinetic model was used to determine the thixotropic constant (k) and the ratio of equilibrium G_e to initial G_0 (G_e/G_0). The k and G_e/G_0 values of the samples were shown in Table 3.11. The k value represents the thixotropic rate of salad dressings, with a larger k showing a higher recovery rate. The k values of salad dressings with higher FOB concentrations were higher. The k value improved when the oil content in sample-1 and sample-2 was decreased by 10%. The k value of sample-2 with 10% more oil content was greater than the k value of sample-1 with 1% more FOB.

¹⁰ G' (Pa): the storage modulus, FOB: cold-pressed flaxseed oil by-product

Table 3. 11 The 3-ITT parameters of salad dressings contained FOB

Sample	FOB	EYP	XG	Oil	k×1000	G _e /G ₀	R ²
1	0.5	3	0.4	20	5.71	1.23±0.02	0.99
2	1.5	3	0.4	10	7.21	1.21±0.00	0.98
3	1.5	3	0.2	20	7.31	1.27±0.00	0.99
4	1.0	3	0.3	15	6.55	1.17±0.01	0.99
5	0.5	3	0.2	20	6.28	1.12±0.01	0.98
6	1.0	3	0.3	20	7.33	1.22±0.01	0.99
7	1.0	3	0.3	15	4.92	1.19±0.00	0.98
8	1.5	3	0.2	10	6.23	1.12±0.02	1.00
9	0.5	3	0.2	10	1.65	1.14±0.01	1.00
10	1.5	3	0.4	20	9.23	1.25±0.00	1.00
11	1.0	3	0.3	10	7.09	1.21±0.04	0.98
12	1.0	3	0.3	15	8.93	1.16±0.00	0.98
13	1.5	3	0.3	15	9.71	1.22±0.00	0.99
14	1.0	3	0.4	15	7.49	1.19±0.03	0.98
15	0.5	3	0.3	15	5.95	1.18±0.01	0.98
16	0.5	3	0.4	10	6.44	1.21±0.00	0.98
17	1.0	3	0.2	15	4.16	1.15±0.01	0.99

¹FOB: cold-pressed flaxseed oil by-product; EYP: egg yolk powder; XG: xanthan gum; k: the thixotropic constant; G_e/G₀: the ratio of equilibrium G_e to initial G₀.

The oil content in the salad dressing determines its rheological properties. As the oil content in a salad dressing is reduced, the rheological properties, particularly the recoverable property, are weakened. It is essential to improve the recoverable characteristic using proper fat replacers, which diminishes as the oil content reduces. In this study, the recovery characteristics of samples with high FOB values were shown to be excellent. Increased intermolecular contacts produced with the formation of small hydrocolloid aggregates may explain the increased recoverability when FOB and XG concentrations increase. These linkages and aggregates can be disrupted by raising the applied shear force in the second interval. When low shear forces are applied during the third time interval, this interaction might form. As this internal structure is created at lower shear stress, the 3-ITT test demonstrates strong thixotropic activity and recoverability [148].

The recovery characteristic of FOB can be recovered with a drop in the gum ratio, similar to the decrease in the oil ratio. As demonstrated in this work, FOB may be utilized to improve the rheological characteristics and thixotropic character of salad dressing.

3.5.6 Determination of optimum formulation

The control samples contained 0.35% XG and 30% oil, and the formulation optimization was based on the lowest oil content and K value of the control samples. 0.3 % XG, 10% oil, and 1.49 % FOB yielded the best results. The addition of 1.49 % FOB with less than 20% oil content demonstrated rheological behavior comparable to control salad dressing. FOB includes polysaccharides, which perform as emulsion stabilizers by forming an enlarged network in the continuous phase and increasing viscosity, as well as proteins, which perform as emulsifiers by adsorbing at the oil-water interface. Cold-pressed flaxseed by-products can therefore be used as a stabilizer and emulsifier.

3.6 The rheological properties of the salad dressing samples contained cold-pressed pumpkin seed oil by-product

In this part of the study, the formulation optimization of salad dressings containing POB was conducted to determine the optimum amount of these by-products. For this purpose, 17 different formulations were prepared with 1.0-5.0% POB. The optimum FOB amount was determined by RSM and CCD.

3.6.1 Steady-shear rheological properties of salad dressing samples contained POB

Figure 3.8 showed that shear rate versus shear stress curves showed the non-Newtonian flow behavior for the salad dressing containing 17 different levels of XG, POB, and oil. The flow behavior of salad dressings is an important parameter in Figure 3.8. As seen in Figure 3.8, all salad dressings exhibited shear-thinning or pseudoplastic behavior because the shear stress of the samples decreased as the shear rate increased.

Lai and Lin [98] were revealed that the low-fat salad dressing model emulsions indicated the shear-thinning flow behavior. This indicates a decrease in the

viscosity values of salad dressing due to the increased shear rate. As previously stated that the reduction in viscosity values of salad dressing type food emulsions may be explained by a significant structural breakdown, which can be related to both an irreversible process (coalescence) and a reversible one (deflocculation) [149-153].

When Figure 3.8 is examined, the first curve that draws attention belongs to the POB-10 sample, and the difference between the curves of the POB-10 sample (30% oil, 0.4% XG, 5% POB) and the POB-2 sample (30% oil, 0.4% XG, 1% POB) and, which is the example below, is very large. The only difference between the two examples is that the amount of POB increased from 1% to 5%. As can be understood from this figure, POB causes a serious increase in consistency in salad dressing products with its high fiber content. When Figure 3.8 is examined, it is seen that POB-3 sample (20% oil, 0.4% XG, 3% POB) and POB-15 sample (10% oil, 0.4% XG, 5% POB) have similar curves. The reason why the curve is similar with decreasing the oil amount from 20% to 10% can be explained by increasing the amount of POB from 3% to 5%.

The decrease of oil content in O/W emulsions (e.g. salad dressing, mayonnaise, etc.) has a significant impact on their rheological and textural behavior, as well as their stability during storage. As a result, the fact that a low-fat salad dressing with POB (although at low concentrations) and a high-fat salad dressing have similar rheological characteristics is significant for the food industry.

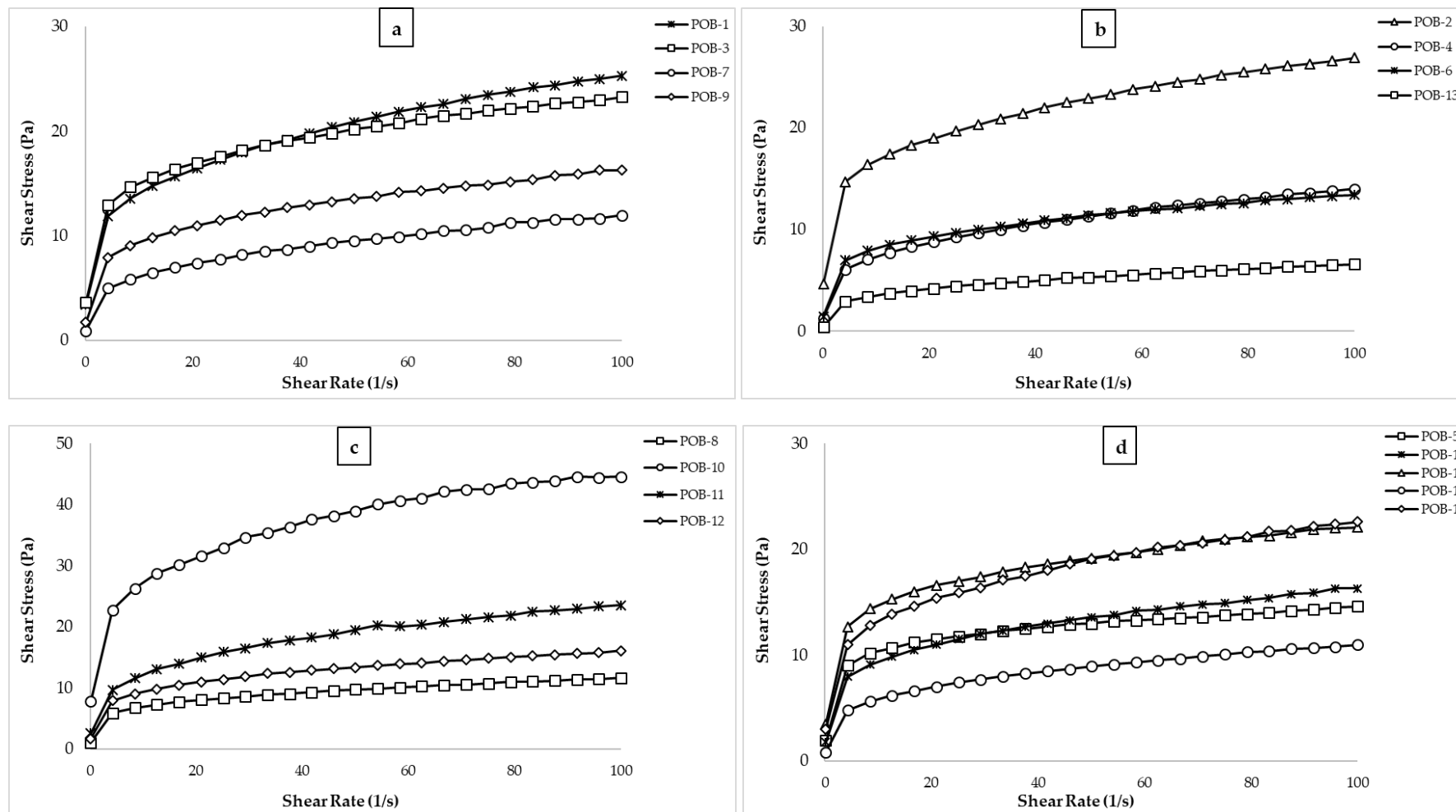


Figure 3. 8 Steady shear rheological properties of the salad dressings contained with POB¹¹

¹¹ POB: Cold-pressed pumpkin seed oil by-product

Power Law model parameters (K and n values) and determination coefficient (R^2) were obtained for 17 different points (Table 3.12). The Power Law model has R^2 values greater than 0.97, indicating that it was acceptable for predicting the flow behavior features of salad dressing samples. Consistency, an important quality characteristic for emulsion-type foods such as purees, pastes, sauces, and salad dressings, indicates a strong interaction between the molecules in the sample structure and stability and shows the thickness of the emulsions [154]. The consistency index (K) values were determined by Eq. 1 and found as between 0.64 Pa s ^{n} and 93.40 Pa s ^{n} (Table 3.12). As can be observed from the table, K increased with increasing XG (g/100g) and/or with increasing POB (g/100g) and lowering oil (g/100g). The K value increased with the increase of POB (g/100g) because the high branched polysaccharide of POB provided the high water-holding ability [19]. Optimization was examined based on the desired K value of 7.5 Pa s ^{n} in a salad dressing with 30% oil (full-fat salad dressing). Considering this value, when the amount of POB was increased from 1% to 5% in POB-5 and POB-15 samples containing 10% oil, the K value increased from 6.41 Pa s ^{n} to 8.95 Pa s ^{n} . With this situation, it was concluded that with the addition of more than 1% POB, low-fat salad dressing could have a consistency index value similar to full-fat salad dressing. These results indicated the effect of POB on the increase of consistency value.

Papalamprou, Doxastakis and Kiosseoglou [155] reported the effect of lupine seed protein isolate varieties on salad dressing emulsion stability. The flow behavior characteristics of salad dressing were investigated by using 1.5% – 4% of two types of lupine seeds. In terms of being close to our study, based on the 1.5% value, the K value of the E type sample of Lupine seed was 9.4 Pa s ^{n} , n value of 0.50, and the K value of the F type sample was found as 1.2 Pa s ^{n} and n value of 0.81.

Table 3. 12 Steady shear power-law parameters of the salad dressings contained POB

	POB (%)	XG (%)	EYP (%)	Oil (%)	K (Pa s ⁿ)	n	R ²
POB-1	3.0	0.3	3.00	30.0	8.60±0.13	0.26±0.00	1.00±0.00
POB-2	1.0	0.4	3.00	30.0	9.86±0.04	0.22±0.00	1.00±0.00
POB-3	3.0	0.4	3.00	20.0	9.09±0.08	0.20±0.00	0.99±0.00
POB-4	1.0	0.2	3.00	30.0	5.60±0.06	0.29±0.00	1.00±0.00
POB-5	1.0	0.4	3.00	10.0	6.41±0.04	0.18±0.00	1.00±0.00
POB-6	1.0	0.3	3.00	20.0	4.60±0.04	0.23±0.00	0.99±0.00
POB-7	3.0	0.2	3.00	20.0	5.05±0.06	0.30±0.00	1.00±0.00
POB-8	3.0	0.3	3.00	10.0	7.84±0.02	0.24±0.00	0.99±0.00
POB-9	3.0	0.3	3.00	20.0	5.12±0.02	0.25±0.00	1.00±0.00
POB-10	5.0	0.4	3.00	30.0	16.11±0.18	0.23±0.00	1.00±0.00
POB-11	5.0	0.2	3.00	30.0	9.56±0.31	0.28±0.01	1.00±0.00
POB-12	3.0	0.3	3.00	20.0	5.16±0.04	0.24±0.00	1.00±0.00
POB-13	1.0	0.2	3.00	10.0	3.75±0.01	0.29±0.00	1.00±0.00
POB-14	3.0	0.3	3.00	20.0	5.14±0.01	0.25±0.00	1.00±0.00
POB-15	5.0	0.4	3.00	10.0	8.95±0.04	0.20±0.00	0.99±0.00
POB-16	5.0	0.2	3.00	10.0	5.95±0.01	0.29±0.01	1.00±0.00
POB-17	5.0	0.3	3.00	20.0	6.54±0.72	0.24±0.01	1.00±0.00

¹POB: Cold-pressed pumpkin seed oil by-product, XG: xanthan gum, EYP: egg yolk powder, K (Pa sⁿ): consistency coefficient, n: the flow behavior index values.

The flow behavior index (n) is related to the mean oil particle size, the oil particle size distribution, and the colloidal character of the continuous phase for food emulsions [98]. The n values for all salad dressings calculated by fitting the data obtained by the Power-law model were less than 1 (the indicative of pseudo-plastic (shear-thinning) and non-Newtonian behavior) and ranged from 0.17-0.81 (Table 3.12). The non-Newton flow characteristics and the low flow behavior

index value (n) (the more viscosity dependence to the shear rate) is the desired flow behavior properties of salad dressings [31]. A value of n approaching 1 indicates a shift towards Newtonian flow behavior. Also, a decrease in the value of n was observed as the consistency coefficient increased as expected. The n value of the samples with a low consistency coefficient was found to be greater. The negative relationship between the K and n values can be explained by increasing the shear-thinning or pseudoplastic flow character and the formation of a tight structure.

3.6.2 The impact of model parameters on K and n values

Figure 3.9 showed the effect of XG, POB, and oil in the salad dressing formulation on K value. As demonstrated in Figure 3.9, an increase in XG, oil, and POB increased the K value of the salad dressings. This effect can be explained by the chemical structure of POB, which comprises high protein and polysaccharides with strong water-holding and surface-active ability. In addition to its stabilizer properties due to polysaccharides, POB proteins may be absorbed in the interface and have surface-active properties. The K value of the salad dressing samples increased as a result of these properties of protein and polysaccharides of POB. Also, the K value significantly increased as the gum content increased, especially in formulations containing a specific xanthan gum content. The synergistic effect of the components used in salad dressing formulation can explain that the K value increased as the content of XG, oil, and POB increased. The quadratic model was used to determine the effect of parameters on the K value of the salad dressings.

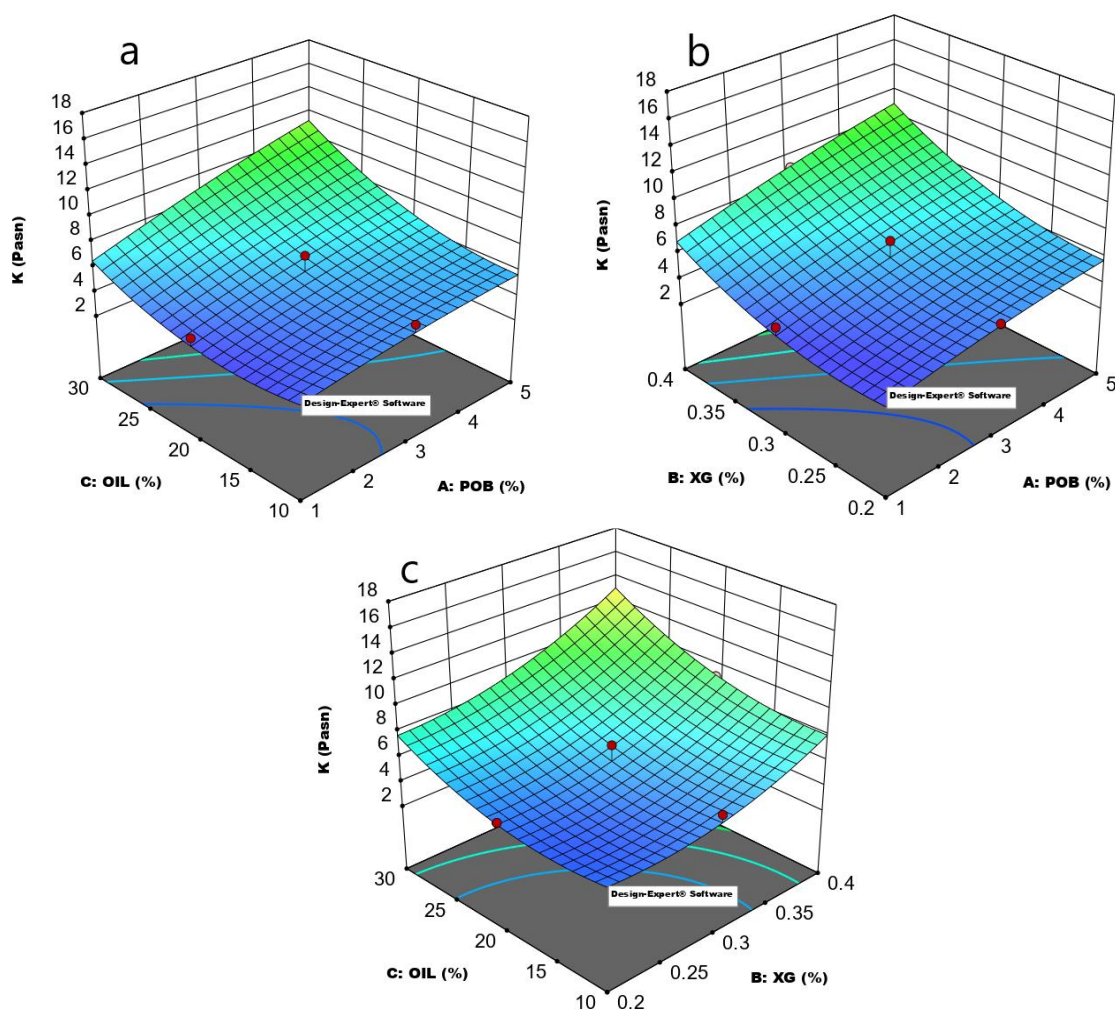


Figure 3. 9 The effect of model parameters on the K value of salad dressing¹²

The model's R^2 , adjusted R^2 , and predicted R^2 values were determined to be 0.9677, 0.9262, and 0.8460, respectively (Table 3.13). The gap between adjusted R^2 and a predicted R^2 was less than 0.2. Therefore, the lack of fit was insignificant. The adequation precision was 18.77. These findings suggested that the quadratic model might be used to accurately characterize the impact of formulation on sample K values. The model's p-value was less than 0.05, indicating that the model terms had a substantial impact on the K value. A, B, C, AC, B², and C² are important model terms in this model. The linear influence of all independent variables had a significant effect. The quadratic effect of B and C, as well as the interaction and model terms A and C, were determined to be significant.

¹² (a): POB-Oil, (b): XG-POB, (c): XG-Oil (A: XG (xanthan gum), B: POB (pumpkin seed oil by-product), C: oil, K: consistency coefficient)

Table 3. 13 The significance of the regression models as a result of RSM

Source	df	K		
		Mean square	F-Value	p-value (Prob > F)*
Model	9	15.36	23.31	0.0002
Linear				
A-POB	1	25.25	38.31	0.0004
B-XG	1	46.27	70.20	< 0.0001
C-Oil	1	39.32	59.67	0.0001
Cross Product				
AB	1	1.65	2.50	0.1579
AC	1	5.23	7.94	0.0259
BC	1	2.15	3.27	0.1137
Quadratic				
A ²	1	0.2030	0.3081	0.5961
B ²	1	4.02	6.10	0.0429
C ²	1	5.06	7.68	0.0276
Lack of Fit	5	0.4052	0.3132	0.8722
R²				0.9677
Adjusted R²				0.9262
Predicted R²				0.8450
Adequation				18.7793
Precision				

¹POB: Cold-pressed pumpkin seed oil by-product, XG: xanthan gum, K, K' & K'' (Pa sⁿ): consistency index values, df: degree of freedom.

²Values of "Prob > F" less than 0.1000 indicate model terms are significant.

3.6.3 The determination of optimum salad dressing formulation contained cold-pressed pumpkin seed oil by-products

The results obtained previous section (3.6.2) showed that POB significantly affected the K value of the salad dressing. The interaction of the POB and oil also be found significant. The optimum formulation was determined as 0.384 % XG, 10 % oil, and 3.04 % POB.

In a study, techno-functional characterization of salad dressings was investigated by adding pulse flour (lentils, chickpeas, and peas) [156]. It was observed that the K value increased with the increase of the unshelled green lentil flour, egg yolk powder, and oil content, and it was observed that the shellless green lentil flour increased the K value more effectively than the egg yolk. The K value increased in the same way with the increase of xanthan gum, oil, and egg yolk components

with shelled green lentil flour, but the main difference between the two was that the 3 independent variables were linear regression for shelled green lentils and non-linear regression for unshelled green lentils.

3.6.4 Dynamic rheological properties

Figure 3.10 shows the results of the dynamic shear test performed to describe the viscoelastic characteristics of the salad dressings and calculate the storage modulus (G') and loss modulus (G''). It was observed that G' values were higher than G'' values in all salad dressings, meaning that all the salad dressings were more elastic than viscous. In the literature, the mayonnaise had a viscoelastic property like salad dressing so that the G' values of mayonnaise were higher than G'' values [157, 158]. The G' values of the samples show solid character [159]. Therefore, when the oil content increases in the salad dressing samples, the G' values of samples increase. As seen in Figure 3.10, all salad dressing samples with higher XG, POB, and oil content indicated a higher G' value. It can be explained that POB enhanced desired solid-like nature of the salad dressings. The dietary fiber content of POB helps to modify the textural properties of the salad dressings, avoid syneresis and stabilize high fat [20].

Ma, Boye, Fortin, Simpson and Prasher [28] reported that all salad dressings were more elastic than viscous in the frequency range, indicating that G'' values were greater than G' values. Also, salad dressings enriched by flour of roasted lentil showed the highest G'' values.

In a study, the effects of starch, xanthan gum, and locust bean gum on the viscoelastic properties of salad dressing samples with reduced fat content were investigated [76]. The researchers reported that the G' and G'' values of the salad dressings increased when the gum concentration increased.

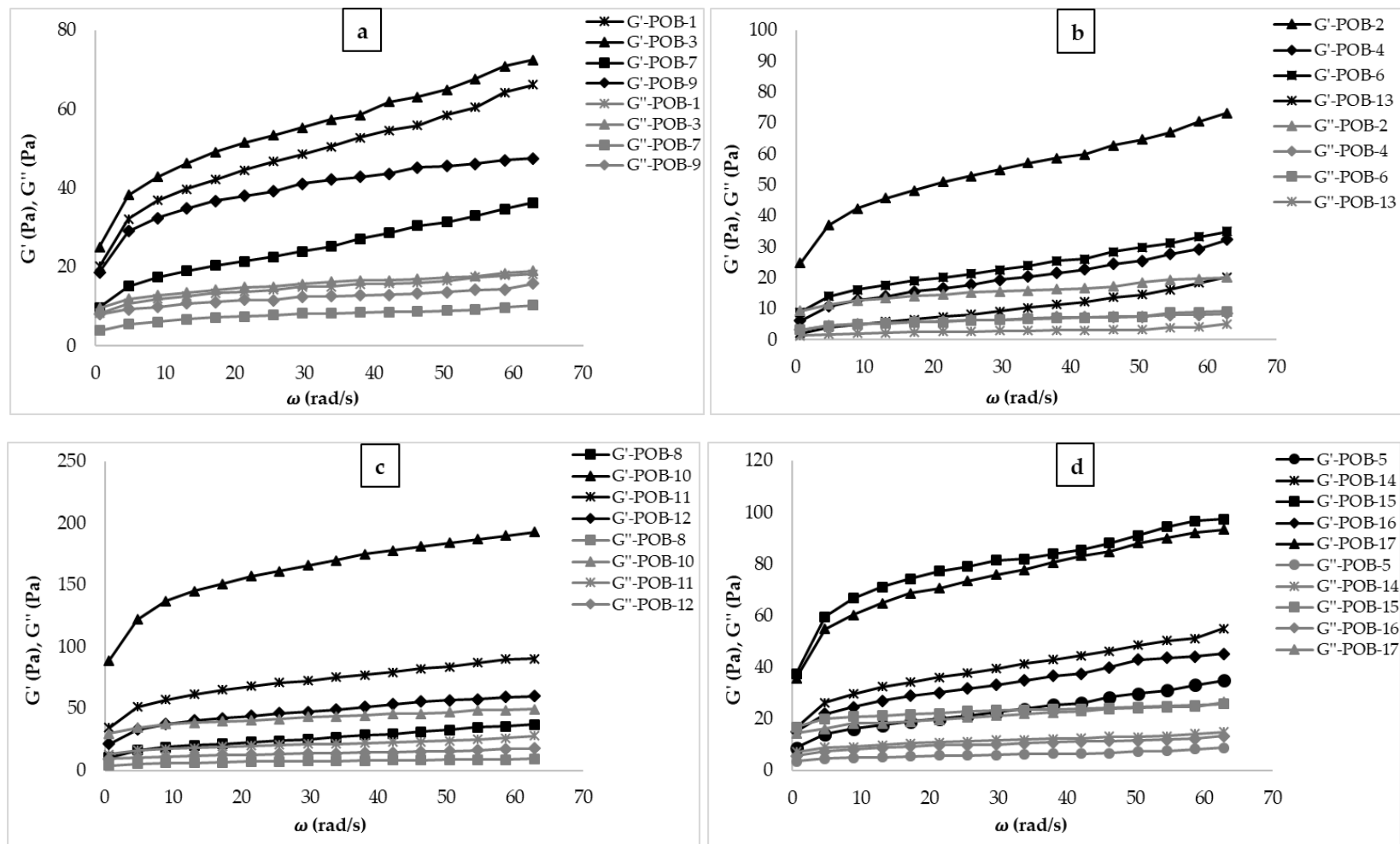


Figure 3. 10 G' and G'' values of salad dressing samples¹³

¹³ POB: Cold-pressed pumpkin seed oil by-product, G' (Pa): storage modulus, G'' (Pa): loss modulus

Table 3. 14 Power-law model parameters of the dynamic rheological properties of the salad dressings

	POB (%)	XG (%)	Oil (%)	$G' = K'(\omega)^{n'}$			$G'' = K''(\omega)^{n''}$		
				K'	n'	R ²	K''	n''	R ²
POB-1	3.0	0.3	30	19.61±0.31	0.28±0.02	0.99±0.00	7.98±0.10	0.19±0.00	0.98±0.00
POB-2	1.0	0.4	30	24.07±0.82	0.25±0.01	0.99±0.01	8.38±0.27	0.20±0.04	0.96±0.02
POB-3	3.0	0.4	20	24.89±0.78	0.25±0.02	0.99±0.00	9.28±0.20	0.16±0.00	0.98±0.00
POB-4	1.0	0.2	30	4.32±0.06	0.46±0.01	0.98±0.01	3.09±0.02	0.23±0.01	0.99±0.00
POB-5	1.0	0.4	10	6.83±0.04	0.37±0.02	0.97±0.01	3.11±0.29	0.22±0.00	0.93±0.03
POB-6	1.0	0.3	20	5.06±0.28	0.42±0.03	0.98±0.00	2.79±0.46	0.27±0.01	0.95±0.02
POB-7	3.0	0.2	20	7.66±0.08	0.36±0.01	0.98±0.01	3.94±0.07	0.22±0.02	0.99±0.00
POB-8	3.0	0.3	10	8.69±0.10	0.34±0.06	0.98±0.01	4.00±0.02	0.20±0.01	0.99±0.00
POB-9	3.0	0.3	20	20.87±0.08	0.20±0.01	1.00±0.00	7.31±0.12	0.16±0.01	0.95±0.02
POB-10	5.0	0.4	30	93.40±2.54	0.17±0.03	1.00±0.00	28.88±1.16	0.12±0.00	0.98±0.01
POB-11	5.0	0.2	30	35.40±0.41	0.22±0.04	0.99±0.00	11.97±0.19	0.18±0.02	0.96±0.02
POB-12	3.0	0.3	20	22.06±0.24	0.24±0.02	0.99±0.00	7.96±0.09	0.17±0.15	0.93±0.03
POB-13	1.0	0.2	10	0.64±0.09	0.81±0.07	0.98±0.01	0.94±0.04	0.35±0.01	0.94±0.05
POB-14	3.0	0.3	20	15.80±0.54	0.28±0.01	0.99±0.00	6.74±0.52	0.17±0.03	0.96±0.02
POB-15	5.0	0.4	10	43.45±1.07	0.18±0.00	0.99±0.00	17.23±0.89	0.09±0.00	0.99±0.00
POB-16	5.0	0.2	10	13.18±0.02	0.29±0.01	0.98±0.01	5.48±0.18	0.20±0.02	0.98±0.00
POB-17	5.0	0.3	20	37.81±0.35	0.21±0.03	0.99±0.00	13.12±0.05	0.15±0.02	0.95±0.02

¹POB: Cold-pressed pumpkin seed oil by-product; XG: xanthan gum; EYP: egg yolk powder (3%); G'(Pa): the storage modulus; G''(Pa): the loss modulus; K', K'': consistency index values, n', n'': the flow behavior index values, ω (s⁻¹): the angular velocity value.

The data of dynamic rheological analysis were obtained by the Power-Law model. K' , K'' , n' , and n'' values were determined by using nonlinear regression (Table 3.14). In Table 3.14, R^2 values were found to be high ($R^2 > 0.93$) and the high value of R^2 shows that the model can successfully explain the dynamic rheological behaviors of the samples. As seen in Table 3.14, K' and K'' values of the salad dressings were estimated as 0.64–93.40 Pa sⁿ, and 0.94–28.88 Pa sⁿ, respectively, and these values changed according to the formulation used.

In a study, the physicochemical properties and stability of reduced-fat salad dressings formulated with pregelatinized potato starch were investigated [160]. The K' values of the salad dressings were found between 0.02–177.0 Pa sⁿ and the K'' values were between 0.08–22.4 Pa sⁿ. The main point affecting the K' and the K'' values is the usage percentages of pregelatinized potato starch (1-5%). When equal to or exceeding 3%, the K' values are above the K'' values, resulting in an example of viscoelastic solid salad dressing.

3.6.5 The 3-ITT rheological properties

Figure 3.11 indicated the 3-ITT rheological properties of the salad dressings. Due to deformations, thixotropic behavior is crucial for O/W emulsions, notably in salad dressings with low oil content. As seen in Figure 3.11, all samples showed thixotropic behavior in the third interval. Samples lost their viscoelastic characteristics after severe shear deformation but recovered them after a second period. These findings suggested that all salad dressing samples may maintain their viscoelastic character throughout food processing involving a large amount of abrupt deformation, such as homogenization or pumping, as well as consumption under shaking and squeezing. This is the ideal flow behavior for salad dressing [75]. Akcicek and Karasu [143] investigated the 3-ITT rheological characteristics of the salad dressing with chia seed oil by-product. In this study, flaxseed oil by-products improved the thixotropic behavior of the salad dressing samples after sudden deformation.

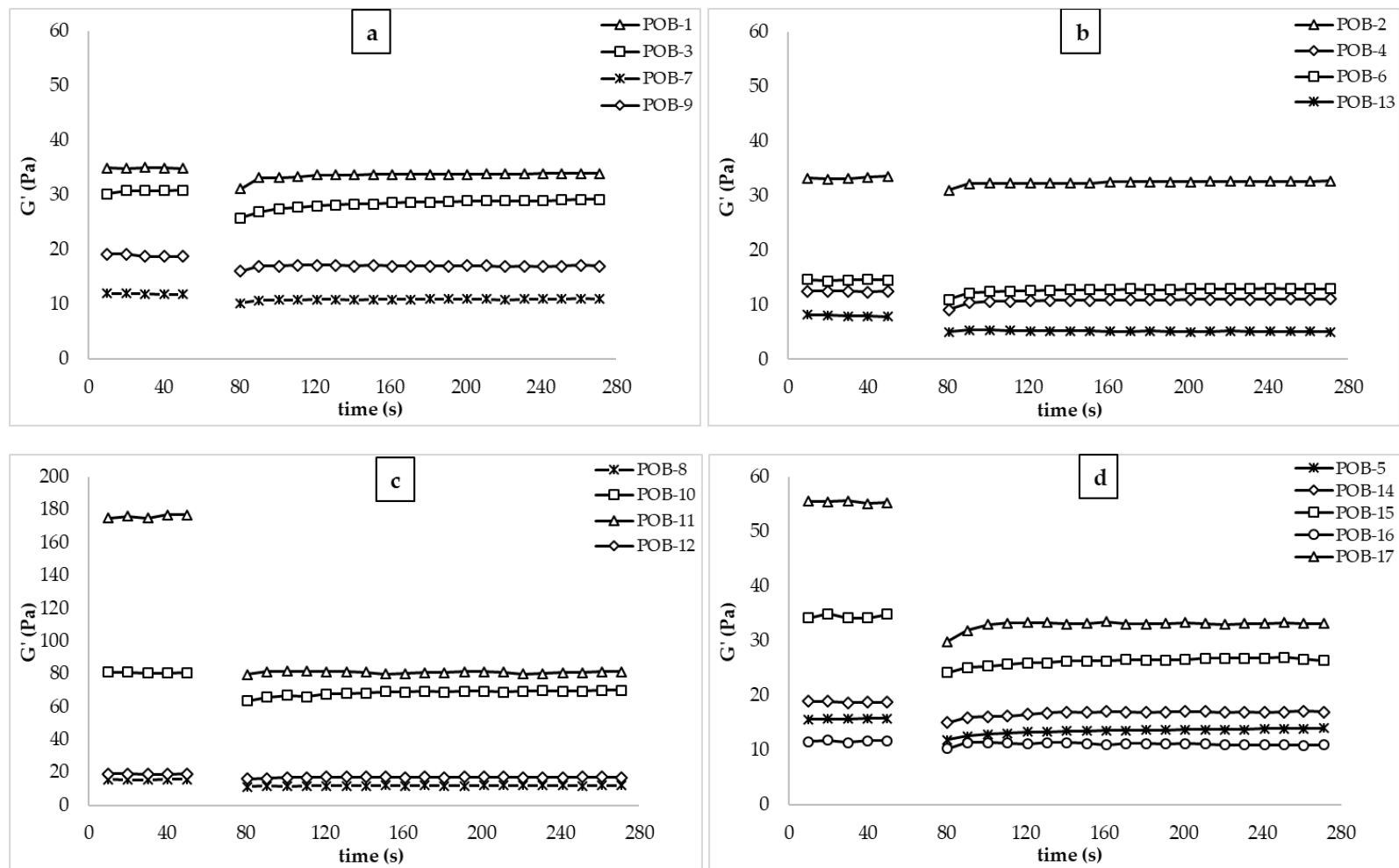


Figure 3. 11 3-ITT rheological properties of salad dressing samples¹⁴

¹⁴ POB: Cold-pressed pumpkin seed oil by-product, G' (Pa): storage modulus

Table 3.15 showed that the second-order structural kinetic model parameters (G_0 , G_e , k) obtained by Eq. 4 and $k \times 1000$ value and the ratio of equilibrium (G_e) and initial (G_0) (G_e/G_0). G_0 , G_e , G_e/G_0 , k , $k \times 1000$, and R^2 values were 5.61-60.19, 5.02-90.85, 0.89-1.51, 0.02-0.13, 9.33-128.55, and 0.95-1.00, respectively. POB-10 sample (30% oil, 0.4% XG, 5% POB) had the highest G_0 , G_e , G_e/G_0 , $k \times 1000$ values, that is, POB-10 indicated the highest thixotropic behavior and viscoelastic solid property. Therefore, the sample showed the highest thixotropic behavior. Akcicek and Karasu [6] found that G_e/G_0 values were between 1.13 and 1.97.

The oil content of O/W emulsions had a significant effect on their rheological properties. As a result, when the oil content of the salad dressings reduced, the rheological characteristics, particularly the recoverable character, decreased. As can be observed in Table 3.15, POB-1 and POB-17 have close G_e/G_0 (1.23 and 1.21, respectively) values, indicating that although the oil is reduced by 10%, increasing the amount of POB from 3% to 5% allows it to exhibit similar thixotropic properties. Therefore, low-fat salad dressings were utilized with POB so that the rheological properties and thixotropic properties of low-fat salad dressings could be enhanced. Increased intermolecular contacts caused by the development of small hydrocolloid aggregates can explain the greater recoverable feature when POB and XG concentrations rise. These interactions and aggregation may be interrupted by increasing the strong shear force given in the second period. This interaction might develop during the third time interval when the applied shear forces are minimal. Forming this internal structure at lower shear stress during the 3-ITT test demonstrates excellent thixotropic behavior and recoverability [148].

Table 3. 15 Second-order structural kinetic model parameters of salad dressings for 3-ITT

	POB (%)	XG (%)	Oil (%)	k	G _e	G ₀	k×1000	G _e /G ₀	R ²
POB-1	3.0	0.3	30.0	0.07±0.01	29.76±0.38	24.22±0.54	67.80	1.23	0.98±0.00
POB-2	1.0	0.4	30.0	0.05±0.03	50.61±2.69	42.23±0.47	53.97	1.20	0.96±0.02
POB-3	3.0	0.4	20.0	0.05±0.00	29.64±0.44	24.88±0.00	52.27	1.19	1.00±0.00
POB-4	1.0	0.2	30.0	0.04±0.00	11.21±0.23	9.67±0.26	41.97	1.16	0.99±0.00
POB-5	1.0	0.4	10.0	0.04±0.01	14.20±0.86	12.71±0.30	36.54	1.12	1.00±0.00
POB-6	1.0	0.3	20.0	0.04±0.02	13.07±0.54	11.67±0.26	37.79	1.12	0.99±0.00
POB-7	3.0	0.2	20.0	0.03±0.00	11.20±1.17	10.47±0.65	27.44	1.07	0.99±0.00
POB-8	3.0	0.3	10.0	0.03±0.03	12.10±0.10	10.90±0.14	34.36	1.11	0.95±0.04
POB-9	3.0	0.3	20.0	0.03±0.00	26.45±0.00	24.11±0.00	33.55	1.10	0.99±0.00
POB-10	5.0	0.4	30.0	0.13±0.01	90.85±1.03	60.19±1.02	128.55	1.51	0.97±0.00
POB-11	5.0	0.2	30.0	0.09±0.02	82.09±2.71	57.24±2.09	87.49	1.43	0.97±0.00
POB-12	3.0	0.3	20.0	0.03±0.00	26.52±0.00	24.40±0.00	30.11	1.09	0.99±0.00
POB-13	1.0	0.2	10.0	0.02±0.00	5.02±0.11	5.61±0.22	23.30	0.89	0.97±0.00
POB-14	3.0	0.3	20.0	0.03±0.00	26.54±0.00	24.32±0.00	31.24	1.09	0.99±0.00
POB-15	5.0	0.4	10.0	0.05±0.00	27.18±1.50	22.61±0.51	54.78	1.20	0.98±0.00
POB-16	5.0	0.2	10.0	0.03±0.00	12.62±0.42	11.64±0.76	29.33	1.08	0.99±0.00
POB-17	5.0	0.3	20.0	0.06±0.04	32.87±0.68	27.21±0.00	57.63	1.21	0.98±0.00

¹POB: Cold-pressed pumpkin seed oil by-product; XG: xanthan gum; EYP: egg yolk powder (3%); k: the thixotropic rate constant; G₀ (Pa): the initial storage modulus in the third time interval; G_e: the equilibrium storage modulus.

3.6.6 The rheological properties of low-fat and high-fat control salad dressings and optimum sample enriched with cold-pressed pumpkin seed oil by-products

The K value of the HF-SD was used to optimize the formulation (30% of oil and 0.35% XG). The steady-shear, dynamic, and thixotropic characteristics of HF-SD, LF-SD, and POBLF-SD were shown in Table 3.16.

The K value of HF-SD, which was chosen as the target value to identify the optimum salad dressing formulation, was determined as 8.02 Pa sⁿ. In this study, the aim was to obtain the optimum low-fat salad dressing (POBLF-SD) by utilizing POB with properties similar to high-fat salad dressing. To identify the optimum formulation, the highest desirability value (1.00) and the low oil content (10%) were chosen as the criterion. The formulation of POBLF-SD was 10% of oil, 0.36% of XG, and 3.04% of POB. We produced optimum (POBLF-SD) and control samples (HF-SD and LF-SD) and compared them to validate the experimental data and RSM results. Table 3.15 showed the steady-shear, dynamic and thixotropic properties of HF-SD, LF-SD, and POBLF-SD. K value of POBLF-SD was found as 8.21 Pa sⁿ, indicating that the correlation between the experimental and predicted values were high and that RSM accurately characterized the optimization process.

The flow behavior of salad dressings could be interpreted through curves of shear stress vs shear rate (Figure 3.12). As can be seen, HF-SD, LF-SD, and POBLF-SD samples showed that shear-thinning rheological properties. The shear-thinning behavior is the typical rheological behavior salad dressings fitted to the power-law model [98, 161, 162]. The K and n values and the R² of the salad dressings were between 3.78-8.21 Pa sⁿ, 0.19-0.23, and 0.99-1.00, respectively (Table 3.16). These parameters of HF-SD and POBLF-SD samples were similar so that POB can be used as a fat substitute for salad dressing samples. This result showed that POBLF-SD and HF-SD (30% oil content) showed similar rheological behavior thanks to the addition of 3.04% POB, although POBLF-SD contained 20% less oil.

Table 3. 16 The rheological properties of HF-SD, LF-SD, and POBLF-SD.

Rheological analysis	Parameters	Samples		
		HF-SD	LF-SD	POBLF-SD
Steady shear $\sigma = K \times \gamma^n$	K	8.02	3.78	8.21
	n	0.21	0.23	0.19
	R ²	1.00	0.99	0.99
Frequency $G' = K' \times (\omega)^{n'}$ $G'' = K'' \times (\omega)^{n''}$	K'	13.80	5.35	15.78
	n'	0.36	0.17	0.14
	R ²	1.00	0.99	0.98
	K''	5.74	1.20	6.16
	n''	0.24	0.37	0.09
	R ²	0.99	0.82	0.93
3-ITT	G ₀	16.74	6.87	17.91
	G _e	20.65	8.00	22.85
	k	0.05	0.04	0.06
	G _e /G ₀	1.23	1.16	1.28
	k×1000	45.01	43.32	56.64
	R ²	0.98	0.98	0.99

¹HF-SD: High-fat salad dressing sample, LF-SD: Low-fat salad dressing sample, POBLF-SD: Low-fat salad dressing sample with cold-pressed pumpkin seed oil by-product (10% oil, 0.365% XG, 3.004% POB), K, K', K'' (Pa sⁿ): consistency index values, n, n', n'': the flow behavior index values; γ (s⁻¹): shear rate; ω (s⁻¹): the angular velocity value; G₀ (Pa): the initial storage modulus in the third time interval; G_e: the equilibrium storage modulus; k: the thixotropic rate constant; R²: Coefficient of determination.

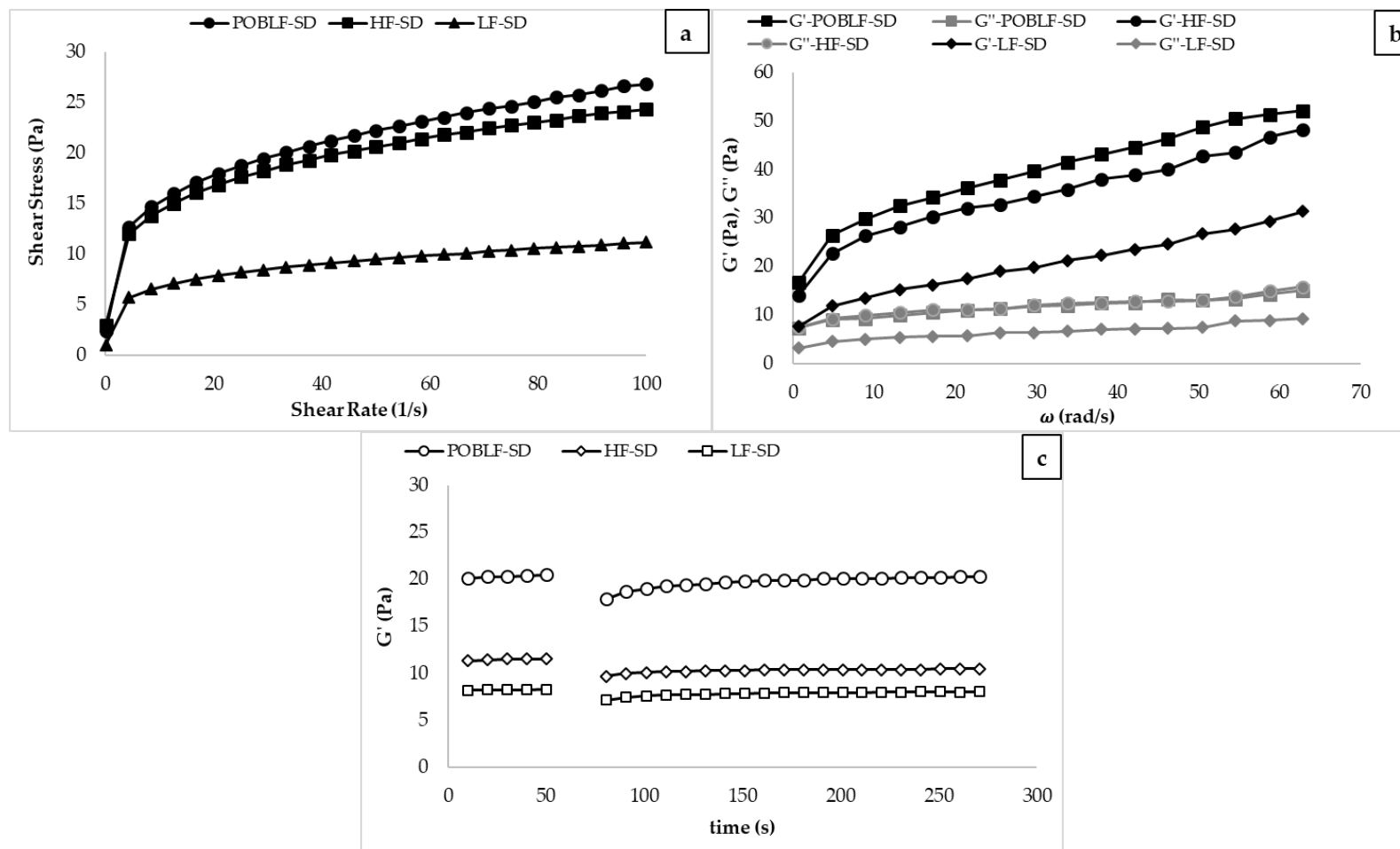


Figure 3. 12 Rheological properties of POBLF-SD, HF-SD, LF-SD¹⁵

¹⁵ HF-SD: High-fat salad dressing sample, LF-SD: Low-fat salad dressing sample, POBLF-SD: Low-fat salad dressing sample with cold-pressed pumpkin seed oil by-product, G' (Pa): storage modulus, G'' (Pa): loss modulus

POB polysaccharides perform as stabilizers in emulsions by creating an extensive network in the continuous phase, increasing viscosity, while POB proteins act as emulsifiers by adsorbing at the oil-water interface. As a result, POB could be used as a stabilizer and emulsifier in low-fat salad dressing samples. In this study, cold-pressed by-products (BOB, COB, FOB, and POB) may be utilized in salad dressings as natural oil replacements, stabilizers, emulsifiers, and functional additives. In this study [163], all by-products (FOB, POB, COB, and BOB) improved the pseudoplastic and viscoelastic solid properties of low-fat salad dressing samples and may be used as a natural oil substitute in low-fat salad dressing at a specific ratio (3% for FOB & POB and 1% for BOB & COB).

The dynamic rheological behavior of salad dressings prepared according to optimum, high-fat, and low-fat salad dressing formulations was shown in Figure 3.12. In salad dressing samples, G' and G'' values of salad dressings increased with increasing frequency. This increase can be evidence of the gel-like property of salad dressings [162]. As seen in Figure 3.12, the G' values of all salad dressings were likewise greater than the G'' value. All salad dressings showed a more solid-like character than the liquid character. Also, the G' value of HF-SD (30% oil) and POBLF-SD (10% oil) were similar, explaining that 20% oil can be compensated with 3.04% POB (Figure 3.12). As can be seen, the LF-SD has the lowest G' and G'' values. The G' and G'' values of the LF-SD (10% oil, 0.35% XG) were lower than the G' and G'' values of the POBLF-SD (10% oil, 0.36% XG, 3.04% POB). As it can be also seen in Table 3.16, POBLF-SD has the elastic modulus value as much as HF-SD with a high-fat content (30%). Synergetic interactions between POB and other ingredients of salad dressings could enhance food quality due to the functional properties of POB. The Power Law model was also used to determine the dynamic rheological parameters (K' , K'' , n' , and n'') (Table 3.15). The R^2 values of the model were between 0.82 and 1.00. As can be observed in Table 3.15, the K' and K'' values of the salad dressings were 5.35-15.78 and 1.20-6.16, respectively, and the n' and n'' values were 0.14-0.36 and 0.09-0.37, respectively. The K' values of all salad dressings were higher than the K'' values. Therefore, all salad dressing samples had a viscoelastic solid property. When POB was added to

the salad dressings, K' and K'' values of POBLF-SD were also likewise greater than the HF-SD salad dressing's values.

Figure 3.12 demonstrated that all salad dressings displayed thixotropic behavior in the third period, indicating that all salad dressings could regain their viscoelastic behavior following high-sudden deformation during food processing. This thixotropic behavior is desirable for salad dressing samples. Akcicek and Karasu [20] examined the thixotropic behavior of salad dressings that contained chia seed oil by-products. They found that recoverable properties in the third period were consistent with our results. Therefore, POB can be also used as a stabilizer for salad dressing samples. Table 3.16 also showed that 3-ITT parameters (G_0 , G_e , k , G_0/G_e) were obtained with the second-order structural kinetic model. G_0 , G_e , k , G_0/G_e , $k \times 1000$, and R^2 values were 6.87-17.91, 8.00-22.85, 0.04-0.06, 1.16-1.23, 43.32-56.64, and 0.98-0.99, respectively. POBLF-SD showed the highest G_0 , G_e , k , G_0/G_e , $k \times 1000$ values, indicating that the POBLF-SD had the highest thixotropic property. The oil contents of O/W emulsions had an important effect on their rheological properties. This result showed that the addition of POB may prevent the changes in the rheological properties caused by the reduction of oil. The greater recoverable behavior observed with the addition of POB can be attributed to increased intermolecular contacts caused by the development of tiny aggregates of hydrocolloid. According to the results, POB can be used to improve the rheological and thixotropic properties of low-fat content salad dressings (10% oil).

3.7 Emulsion stability, zeta potential, particle size, and microstructure of high-fat and low-fat salad dressing samples and low-fat salad dressing enriched with by-products

Emulsion stability is one of the most significant quality properties of salad dressings [121]. In salad dressings with low emulsion stability, phase separation can be observed on the surface during storage. As explained before, this situation is related to the formulation and process conditions, especially the emulsifier and stabilizer in the structure of the salad dressing.

The thermal loop test was developed to measure emulsion stability. In this study, this test was utilized for the determination of the salad dressing stability. By applying emulsions to varying numbers of heat cycles, the thermal loop test might be utilized as a quick technique to assess emulsion stability. By the thermal loop test, the temperature fluctuations were simulated during the processing, production, storage, and transportation. The cycle-to-cycle variation of modules (G^* , G') was identified by structural or morphological changes induced by applied thermal stress. Figure 3.13-a&b indicated the change in G^* value after thermal stress. As can be observed, a small rise in the G^* value of all salad dressings was obtained after 10 heat cycles. This insignificant change showed that all salad dressings were resistant to thermal stress and had high emulsion stability. This could indicate that the salad dressings containing POB showed the highest stability, followed by BOB.

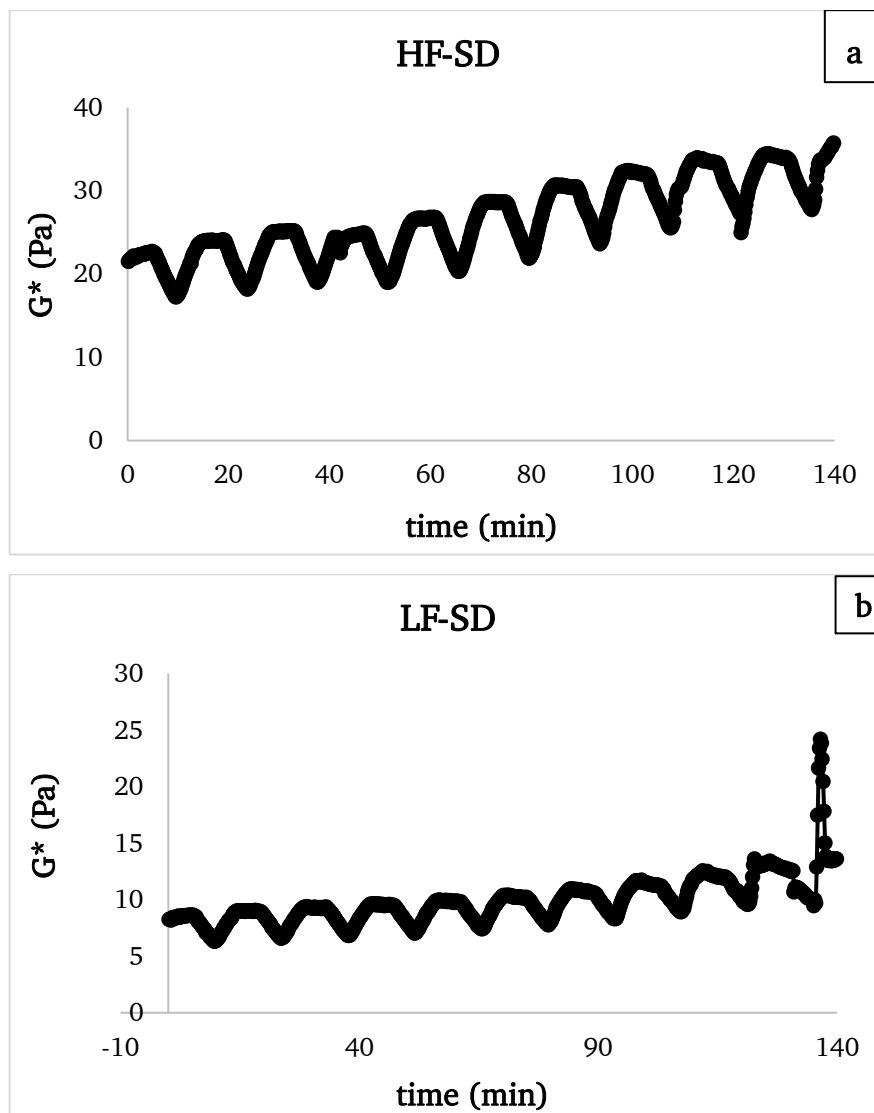


Figure 3. 13- a The change in G^* value after applying the thermal loop¹⁶

¹⁶ HF-SD: High-fat salad dressing sample, LF-SD: Low-fat salad dressing sample

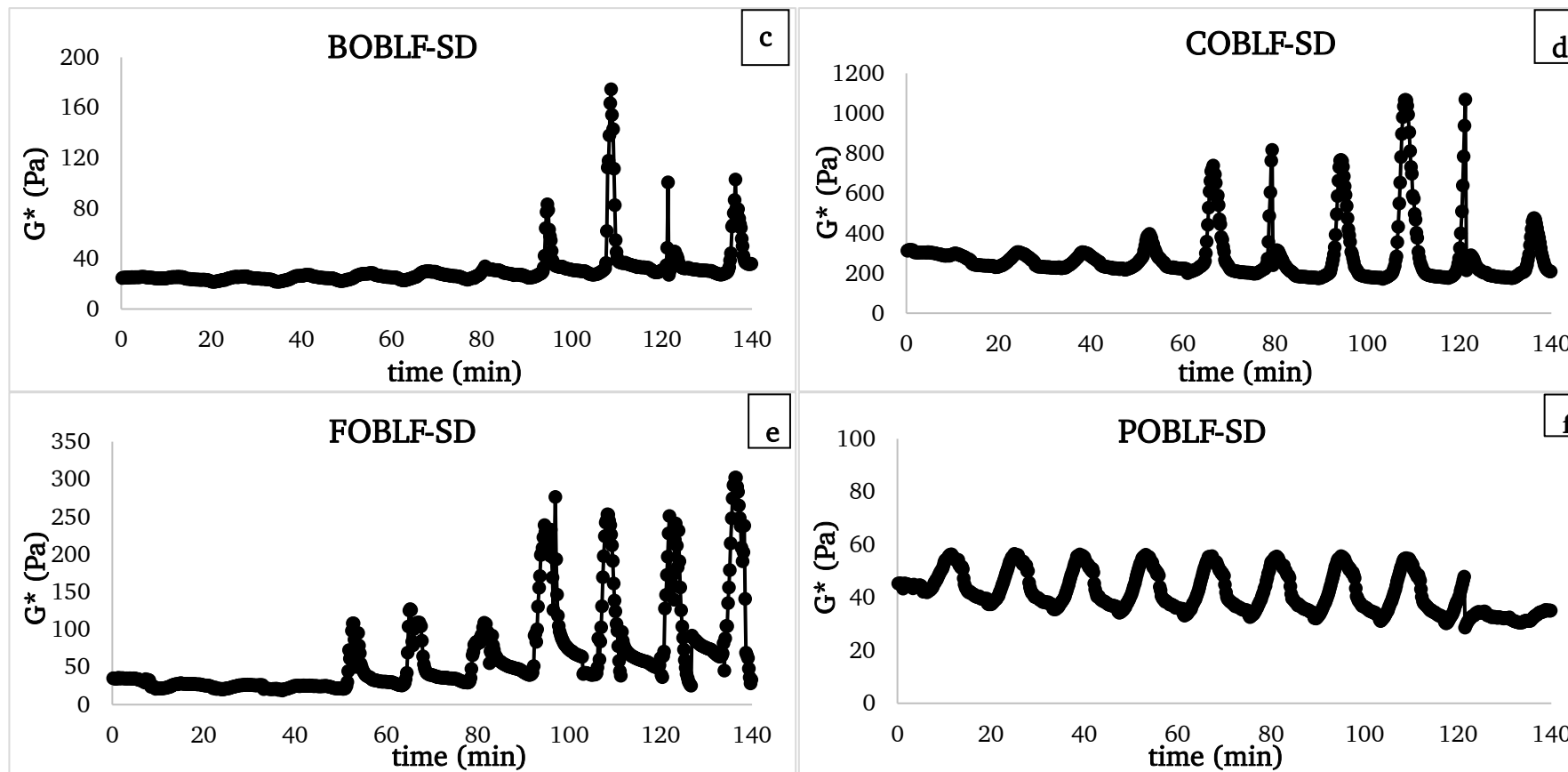


Figure 3. 13- b The change in G^* value after applying the thermal loop ¹⁷

¹⁷ BOBLF-SD: Low-fat salad dressing sample with cold-pressed blackcumin oil by-product, COBLF-SD: Low-fat salad dressing sample with cold-pressed coconut oil by-product, FOBLF-SD: Low-fat salad dressing sample with cold-pressed flaxseed oil by-product, POBLF-SD: Low-fat salad dressing sample with cold-pressed pumpkin seed oil by-product, G^* (Pa): complex modulus

Zeta (ζ) potential is an important parameter that shows whether salad dressings can remain stable for a long time. As the ζ -potential value moves away from 0, in other words, the system having a negative or positive charge is a positive indicator for the long stability of the product. Table 3.17 showed that the ζ -potential values of the samples were found between (-43.15) and (-39.49) mV. The first interpretation we could make by looking at these values is that the ζ -potential value of all samples is higher than 0 or that the samples are stable products to a certain degree. Another important situation was that the absolute value of the ζ -potential of COBLF-SD and POBLF-SD was close to the ζ -potential of HF-SD.

The high ζ -potential value of our salad dressing samples produced in optimum formulations indicated that the optimization product could remain stable in these formulations for a long time. The fact that xanthan gum forms a compact structure by reducing the mobility of the mobile phase in increasing the stability of salad dressing samples, thus reducing the action potential of the oil molecules in this tight structure and restricting the interaction of the droplets play a primary role [164]. However, the interaction of egg yolk and xanthan gum at the interface is related to the charge balance of the medium, in other words, the ζ - potential [165]. The fact that the oil droplets have a certain electrical potential and interact with each other thanks to the electrostatic repulsive force, preventing flocculation. This charge comes from protein and polysaccharides, especially ionizable emulsifiers localized around the oil droplets [33]. These charged structures that can be ionized at the interface have an important role in the stability of the product. Because the egg yolk has a positive charge and the xanthan gum has a negative charge, electrostatic interaction between the two has allowed the formation of a stable structure as a result. Zhao Zhao, Long, Kong, Liu, Sun-Waterhouse and Zhao [147] found a similar result to the results we found in their study. In their research, it was observed that as the gamut ratio increased, the product shifted towards the negative charge in the ζ -potential (-28.05 mV). The researchers stated that this result was due to negatively charged proteins and polysaccharides attached around the oil droplets. The negatively charged proteins around the droplets, thanks to hydrogen bonds and other hydrophobic bonds, the

bond they formed with polysaccharides allowed the formation of a significant negative charge around the droplet.

The oil particle size (d_{32}) and PDI value of the samples were found as 3125.67-5196.00 μm , and 0.27-0.90, respectively (Table 3.17). The researchers emphasized that the oil particle diameter decreased significantly as the polysaccharide concentration increased [71, 166]. As can be seen, the sample containing BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD showed lower particle size and PDI value as compared to HF-SD. These results are consistent with the thermal loop test. As the water ratio increased, the ζ -potential values of the salad dressings decreased. However, all salad dressings had a sufficient zeta potential value.

Table 3. 17 The zeta potential values, particle size, and polydisperse index of the salad dressings

Sample	ζ -potential (mV)	d_{32} (μm)	PdI
HF-SD	-43.15 ± 0.93^a	4904.83 ± 143.01^b	0.90 ± 0.10^a
LF-SD	-39.68 ± 0.75^b	5196.00 ± 65.87^a	0.27 ± 0.07^d
BOBLF-SD	-39.49 ± 0.36^b	3961.75 ± 96.19^c	0.59 ± 0.02^c
COBLF-SD	-43.00 ± 0.97^a	3260.60 ± 81.36^d	0.65 ± 0.02^b
FOBLF-SD	-39.70 ± 0.50^b	4179.83 ± 169.71^c	0.67 ± 0.02^b
POBLF-SD	-42.32 ± 0.68^a	3125.67 ± 32.79^d	0.65 ± 0.08^b

¹HF-SD: High-fat salad dressing sample, LF-SD: Low-fat salad dressing sample, BOBLF-SD: Low-fat salad dressing sample with cold-pressed black cumin oil by-product, COBLF-SD: Low-fat salad dressing sample with cold-pressed coconut oil by-product, FOBLF-SD: Low-fat salad dressing sample with cold-pressed flaxseed oil by-product, POBLF-SD: Low-fat salad dressing sample with cold-pressed pumpkin seed oil by-product, ζ -potential (mV): zeta potential, d_{32} : oil particle size, PDI: polydisperse index.

²Different lowercase letters in the same line represent the differences between samples subjected to different drying methods ($p < 0.05$).

Figure 3.14-a&b showed the oil particle distribution. The HF-SD sample and BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD had homogeneous droplet distribution and low oil droplet size, indicating that the use of cold-pressed oil by-products could have a positive impact on the oil droplet size and distribution. Therefore, emulsion stability in low-fat salad dressing could be enhanced by the addition of the cold-pressed oil by-products.

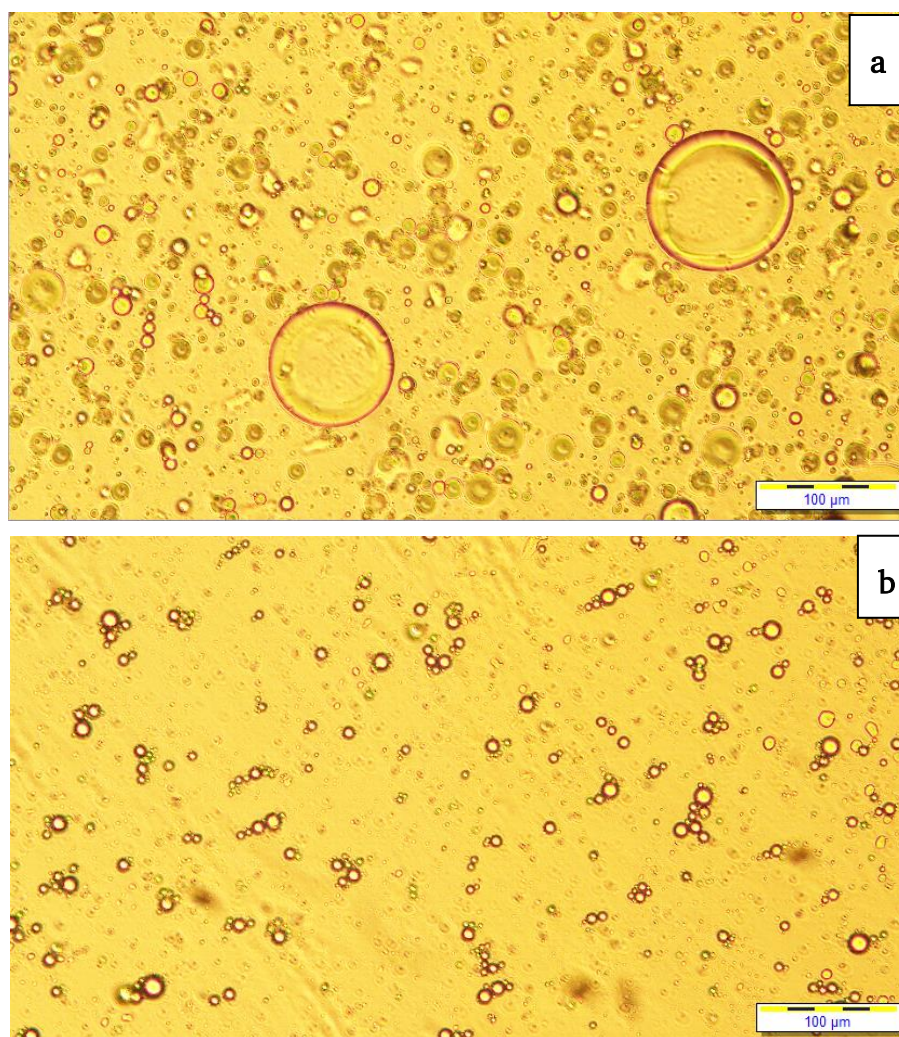


Figure 3. 14- a Light microscopy pictures of HF-SD (a), LF-SD (b)¹⁸

¹⁸ HF-SD: High-fat salad dressing sample, LF-SD: Low-fat salad dressing sample

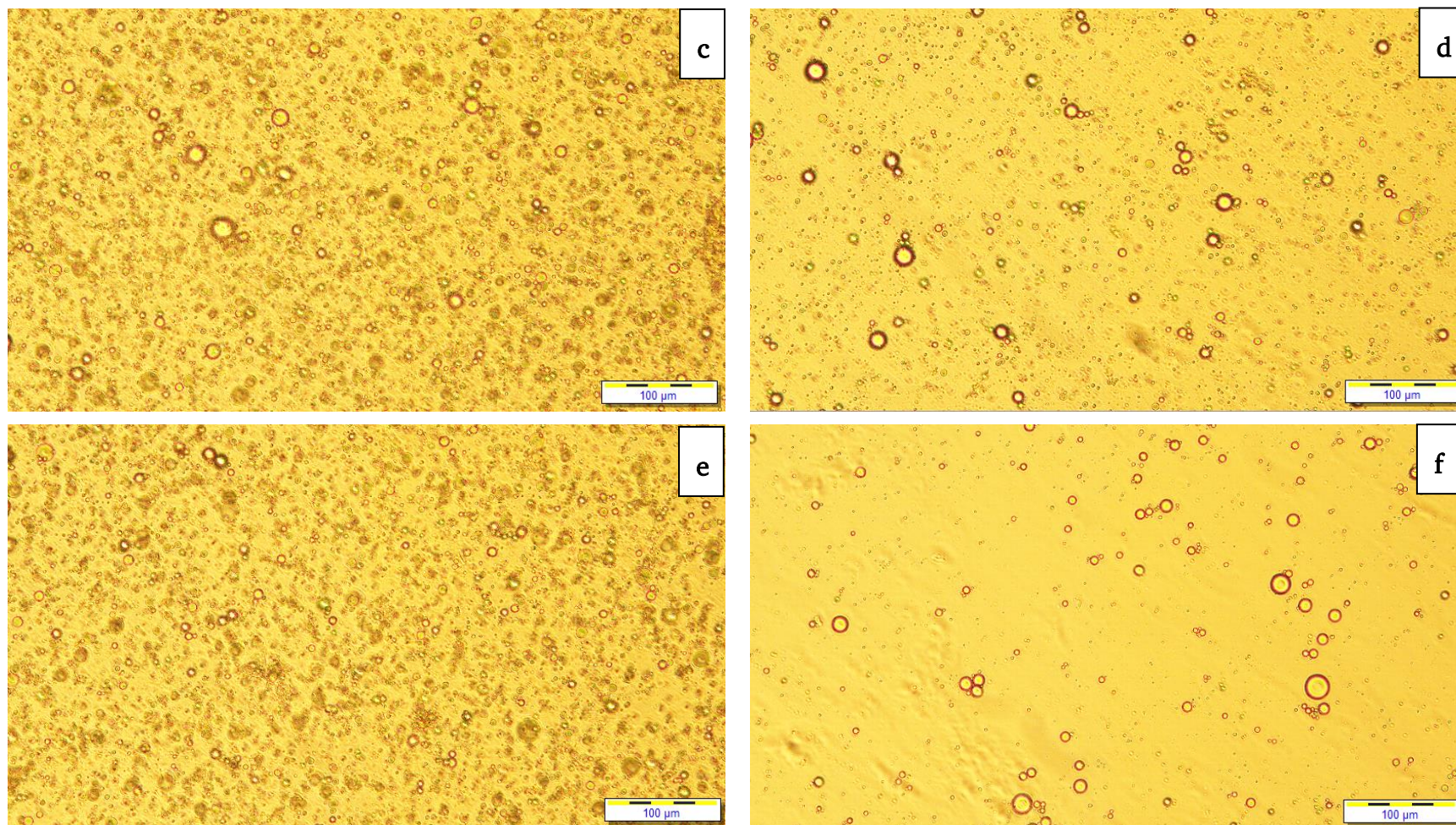


Figure 3. 14- b Light microscopy pictures of BOBLF-SD (c), COBLF-SD (d), FOBLF-SD (e), POBLF-SD (f)¹⁹

¹⁹ BOBLF-SD: low-fat salad dressing enriched with BOB; COBLF-SD: low-fat salad dressing enriched with COB; FOBLF-SD: low-fat salad dressing enriched with FOB; POBLF-SD: low-fat salad dressing enriched with POB

3.8 Oxidative stability of salad dressing samples enriched with by-products

The IP values of HF-SD, LF-SD, BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD were shown in Table 3.18. The IP values of HF-SD, LF-SD, BOB, COB, FOB, and POB samples were found as 1.03-12.57 h, 0.45-10.34 h, 1.18-14.06 h, 0.56-16.02 h, 1.31-15.10 h, and 1.29-16.25 h, respectively. The HF-SD sample showed a higher IP value than the LF-SD sample, indicating that the decrease in the oil ratio in salad dressing led to a decrease in the IP value. BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD showed a higher IP value than HF-SD and LF-SD, indicating that BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD showed the highest oxidative stability.

Arrhenius and Eyring's equations utilized nonlinear regression to predict the influence of temperature on the oxidation kinetics of the samples. The Arrhenius and Activation's complex parameters were reported in Table 3.18. As demonstrated, sample type had a significant impact on E_a , ΔH^{++} , ΔS^{++} , and ΔG values ($p < 0.05$). The E_a value shows the minimum energy value required to initiate oxidation. E_a values were 87.63, 76.69, 95.81, 122.26, 95.38, and 95.97 kJ/mol for HF-SD, LF-SD, BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD, respectively, implying that the E_a values of the samples contained by-products compared to LF-SD significantly raised by adding by-products. Aksoy, Tekin-Cakmak, Karasu and Aksoy [122] reported that the addition of some microencapsulated phenolic extracts significantly increased the E_a value of the salad dressings. According to Farhoosh [167], the type of antioxidant differently affected E_a of the oil oxidation.

Table 3. 18 The oxidation kinetic parameters of the salad dressing samples.

Sample	T (°C)	IP (h)	E _a (kJ/mol)	ΔH ⁺⁺ (kJ/mol)	ΔS ⁺⁺ (J/mol/K)	ΔG ⁺⁺ (kJ mol ⁻¹)
HF-SD	80	12:57	86.63	89.38	6.61	87.05
	90	3:20				86.98
	100	1:55				86.92
	110	1:03				86.85
LF-SD	80	10:34	76.69	94.53	22.35	86.64
	90	2:58				86.41
	100	1:27				86.19
	110	0:45				85.97
BOBLF-SD	80	14:06	95.81	87.76	-14.13	92.75
	90	9:56				92.89
	100	3:10				93.03
	110	1:18				93.18
COBLF-SD	80	16:02	122.26	112.80	59.10	91.93
	90	5:16				91.35
	100	2:24				90.75
	110	0:56				90.16
FOBLF-SD	80	15:10	95.38	87.35	-15.73	92.91
	90	10:02				93.06
	100	3:08				93.22
	110	1:31				93.38
POBLF-SD	80	16:25	95.97	77.86	-28.84	88.04
	90	6:20				88.33
	100	2:46				88.62
	110	1:29				88.91

¹HF-SD: High-fat salad dressing sample; LF-SD: Low-fat salad dressing sample; BOBLF-SD: Low-fat salad dressing sample with cold-pressed black cumin oil by-product; COBLF-SD: Low-fat salad dressing sample with cold-pressed coconut oil by-product; FOBLF-SD: Low-fat salad dressing sample with cold-pressed flaxseed oil by-product; POBLF-SD: Low-fat salad dressing sample with cold-pressed pumpkin seed oil by-product; T: Temperature (°C); IP: induction period (h); E_a: Activation energy (kJ/mol); ΔH⁺⁺: Activation enthalpy (kJ/mol); ΔS⁺⁺: Entropy change (J/mol/K).

The activation complex parameters, namely, ΔH^{++} , ΔG^{++} , and ΔS^{++} values were examined for the evaluation of the influence of temperature on the oxidation kinetic of salad dressings (Table 3.18). ΔH^{++} values of the HF-SD, LF-SD, BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD were calculated as 89.38, 94.53, 87.76, 112.80, 87.35, and 77.86 kJ/mol, respectively, while the ΔS^{++} value was found as 6.61, 22.35, -14.13, 59.10, -15.73, and 28.84 J/mol/K, respectively. For salad dressing and oil oxidation, similar ΔH^{++} and ΔS^{++} values were found as in the literature [168-170]. As seen in Table 3.18, the low-fat and high-fat control samples (LF-SD and HF-SD) had positive ΔS^{++} values, but the BOBLF-SD, FOBLF-SD, and POBLF-SD had negative ΔS^{++} values. The endothermic nature of activated compound formation is indicated by a positive value of ΔH^{++} . As seen by the large negative value of ΔS^{++} , the activated complexes are more structured than the reactants compounds, with lesser species in the activated complex state [169]. The negative value of ΔS^{++} obtained from the salad dressings containing BOBLF-SD, FOBLF-SD, and POBLF-SD indicated a slower oxidation rate. The decrease in free radical concentrations caused by the hydrogen donation of the antioxidants of BOBLF-SD, FOBLF-SD, and POBLF-SD and the reduction of rotational flexibility in the transiently activated complex can be explained by the drop in ΔS^{++} with the addition of BOBLF-SD, FOBLF-SD, and POBLF-SD [168]. However, COBLF-SD had a lower TPC value, explaining that COBLF-SD had a positive ΔS^{++} value.

ΔG^{++} is the free activation energy that provides quantitative information about the rate of oxidation. The higher values of ΔG^{++} , the slower the oxidation rate, and the greater the oxidation stability. ΔG^{++} values of HF-SD, LF-SD, BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD samples were 86.85-87.05 kJ/mol, 85.97-86.64 kJ/mol, 92.75-93.18 kJ/mol, 90.16-91.93 kJ/mol, 92.91-93.38 kJ/mol, and 88.04-88.91 kJ/mol, respectively, indicating that the salad dressing contained FOB showed the highest ΔG^{++} value, followed by BOBLF-SD, COBLF-SD, and POBLF-SD. The results of the IP value, the Arrhenius, and the Activation of complex parameters were compatible with each other. These results indicated that the inclusion of BOB, COB, FOB, and POB could improve the oxidative stability and rheological properties of low-fat salad dressing samples. The free radical scavenging activity of the antioxidants of BOB, COB, FOB, and POB, which is

localized to the oil-water interface, can explain the increase in oxidative stability caused by BOB, COB, FOB, and POB addition.

Aksoy, Tekin-Cakmak, Karasu and Aksoy [122] studied whether microencapsulated phenolic extracts of cold-pressed pomegranate seed oil by-product (PGOB) and grape seed oil by-product (GOB) might be used as natural antioxidants in salad dressing formulations. In this study, oxidative stability of the salad dressing stabilized with GOB and PGOB was determined using OXITEST. These microencapsulated phenolic extracts considerably increased IP and ΔG^{++} while decreasing ΔS^{++} and ΔH^{++} values. GOB and PGOB extracts should work well as a natural antioxidant agents in salad dressings and emulsions.

3.9 Conclusion and Suggestions

The physicochemical and bioactive properties of flaxseed, pumpkin, black cumin seed, and coconut by-products were investigated in this analysis to see whether they could be used as useful ingredients in low-fat salad dressing. Cold-pressed oil by-products may be considered a good source of protein and carbohydrates. The quantity and distribution of specific phenolics, as well as TPC and CUPRAC values, differed significantly by type of by-product, with BOB and FOB being particularly rich in phenolic compounds that should be evaluated in food emulsions to develop oxidative stability and increase health benefits. Flow behavior and viscoelastic rheological properties were used to examine the potential application of by-products as natural fat alternatives in a low-fat salad dressing. Except for those enriched with 1% FOB and POB, salad dressings contained by-products that demonstrated shear-thinning and viscoelastic solid characteristics. By-products could be employed as natural fat replacers and useful ingredients in a low-fat salad dressing, according to results in this study.

The salad dressing samples' FOB content had a significant influence on their rheological, microstructural, recoverable, and stability qualities. A low-fat salad dressing's rheological, microstructural, and recoverable qualities were improved by FOB. All of the salad dressing samples had shear-thinning and viscoelastic solid nature. Salad dressing prepared with low oil and high FOB content had similar steady-state and viscoelastic flow behavior to salad dressing formulated with high

oil content. These studies demonstrated that salad dressing samples produced with low oil and high FOB content had a positive recoverable character and could keep their viscoelastic nature during a high-sudden-deformation food process. FOB could be employed as a natural fat replacer and stabilizer in several food applications, according to the conclusions of this investigation.

The potential use of POB as a fat substitute was also investigated that can be used to obtain low-fat salad dressing with similar rheological, microstructural, recoverable properties, and emulsion and oxidative stability to high-fat salad dressing. The addition of POB considerably contributed to these properties of the salad dressing due to being rich in protein and carbohydrates. All samples with 17 different formulations exhibited shear-thinning and viscoelastic solid behavior. Shear-thinning and viscoelastic behavior of the salad dressing formulated with low oil and POB content (10% oil, 0.3% XG, 3% POB) showed similar characteristics with high oil content salad dressing. These results indicated that salad dressing samples formulated with low oil and 3% POB content showed desirable recoverable character and could preserve their viscoelastic behavior during the process with a high sudden deformation. The low-oil salad dressing containing POB (POBLF-SD), produced as a result of the optimization. POBLF-SD showed similar rheological properties to the high-oil salad dressing (HF-SD).

The thermal loop test was used to determine the emulsion stability. COB showed the highest emulsion stability, followed by BOB. Also, the zeta potential values, oil particle size (d_{32}), and PDI value of the BOBLF-SD, COBLF-SD, FOBLF-SD, and POBLF-SD and control samples (HF-SD and LF-SD) were determined. The adsorption of negatively charged polysaccharides onto the negatively charged proteins' surfaces via various interactions such as H-bonding and hydrophobic contact resulted in higher negative zeta potential. Salad dressing samples with high zeta potential values may have been anticipated to have long-term stability. These findings were reported as consistent with the thermal loop test. By the light microscopy, the oil particle distribution BOBLF-SD, COBLF-SD, FOBLF-SD, POBLF-SD, HF-SD, and LF-SD. The addition of cold-pressed oil by-products could have a positive impact on the oil droplet size and distribution. Also, the emulsion stability of low-fat salad dressings could enhance with the by-products. The

oxidative stability of salad dressings was determined by OXITEST. The bioactive components of BOB, COB, FOB, and POB might have improved the oxidative stability of salad dressing. This study suggested that BOB, COB, FOB, and POB could be successfully used as a natural fat replacer, stabilizer, and oxidative agent in a variety of food applications.

The results of our study showed that cold-pressed oil by-products are rich in protein, carbohydrates, and bioactive substances. In future studies, protein extraction from these by-products and bioactive and technological characterization of the obtained proteins should be investigated. One of the other remarkable features of by-products is their thickening properties. By extracting gum from these by-products, the potential of using these gums as natural stabilizers and encapsulation agents should be investigated. In addition, the extraction of phenolic compounds, lignans, and other antioxidants from these by-products and their potential for use in various foods should be investigated. One of the important suggestions of this study is the ethanol content in by-products. This result indicates yeast fermentation in by-products. The results of our study suggest that cold-pressed oil producers should store by-products after a preservation process such as drying. In addition, the effects of different drying methods and parameters on the bioactive compound properties of by-products can be studied.

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PUBLICATIONS FROM THE THESIS

Conference Papers

1. Z. H. Tekin, and S. Karasu, “Investigation of Potential Use of By-products from Cold-pressed Industry as Natural Fat Substitutes and Functional Ingredients in a Low Fat Salad Dressing”, International Conference on Agronomy and Food Science and Technology, 20-21 June 2019, DoubleTree by Hilton, Avcılar, Istanbul, Turkey, (oral presentation).
2. Z. H. Tekin, and S. Karasu, “The Effects of Some Cold-pressed Oil By-Products on The Rheological, Antimicrobial and In-Vitro Bio-Accessibility Properties of Low Fat O/W Emulsions”, INTERNATIONAL CONGRESS OF FOOD, AGRICULTURAL AND VETERINARY SCIENCES, 29 February - 1 March 2020, Konya, Turkey (oral presentation).

Articles

1. Z. H. Tekin, and S. Karasu, “Cold-pressed Flaxseed Oil By-product as a New Source of Fat Replacers in Low-Fat Salad Dressing Formulation: Steady, dynamic and 3-ITT Rheological Properties”, *Journal of Food Processing and Preservation*, 44:e14650, 2020.
2. Z. H. Tekin-Cakmak, S. Karasu, S. Kayacan-Cakmakoglu, and P. K. Akman, “Investigation of Potential Use of By-products from Cold-pressed Industry as Natural Fat Substitutes and Functional Ingredients in a Low Fat Salad Dressing”, *Journal of Food Processing and Preservation*, e15388, 2021.
3. Z. H. Tekin-Cakmak, I. Atik, and S. Karasu, "The Potential Use of Cold-pressed Pumpkin Seed Oil By-Products in a Low-Fat Salad Dressing: The Effect on Rheological, Microstructural, Recoverable Properties, and Emulsion and Oxidative Stability," *Foods*, vol. 10, no. 11, p. 2759, 2021.