เรื่อง: การสกัดและทำให้บริสุทธิ์ trans-resveratrol จากกากองุ่น พร้อมเพิ่มการนำไปใช้ได้ทางชีวภาพด้วยเทคโนโลยีการห่อหุ้ม
ตีพิมพ์ในวารสาร *Food Chemistry* ปี 2025

ที่มาและวัตถุประสงค์

กากองุ่นจากอุตสาหกรรมไวน์และน้ำองุ่นประกอบด้วยเปลือก เมล็ด และก้าน ซึ่งยังมีสารพอลิฟีนอลที่มีมูลค่าสูง โดยเฉพาะ **trans-resveratrol** สารกลุ่ม stilbene ที่มีฤทธิ์ต้านอนุมูลอิสระและมีศักยภาพต่อสุขภาพหลายด้าน

อย่างไรก็ตาม trans-resveratrol มีข้อจำกัดสำคัญ ได้แก่

- ละลายน้ำได้น้อย
- ไม่เสถียรต่อแสง ความร้อน และออกซิเจน
- เปลี่ยนจากรูป trans เป็น cis ได้ง่าย
- ถูกเมแทบอลิซึมอย่างรวดเร็ว ทำให้ชีวปริมาณออกฤทธิ์ต่ำ

งานวิจัยจึงมีเป้าหมายเพื่อสกัดและทำให้ trans-resveratrol จากกากองุ่นบริสุทธิ์ และห่อหุ้มด้วย **เลซิทิน** เพื่อเพิ่มความเสถียร การละลาย และการปลดปล่อยในระบบทางเดินอาหาร

วิธีการศึกษา

1. การเตรียมวัตถุดิบ

ใช้กากองุ่นจากโรงงานไวน์ใน 3 พื้นที่ของประเทศตุรกี นำมาทำความสะอาด ลดความชื้นให้ไม่เกิน 5% และบดให้มีขนาดประมาณ 40–60 mesh

2. การเปรียบเทียบวิธีสกัด

ทดลองวิธีต่าง ๆ ได้แก่

- การสกัดด้วยตัวทำละลายแบบดั้งเดิม
- การสกัดในสภาวะกรด
- การสกัดแบบรีฟลักซ์
- การสกัดด้วยคลื่นอัลตราโซนิก
- การไฮโดรไลซ์ด้วยกรด
- การสกัดแบบของเหลว–ของเหลว

ตรวจวัด trans-resveratrol ด้วย HPLC-PDA ที่ความยาวคลื่นประมาณ 305 นาโนเมตร

3. การทำให้บริสุทธิ์และยืนยันโครงสร้าง

เปรียบเทียบการแยกด้วย

- คอลัมน์ซิลิกาเจล
- Sephadex LH-20
- Flash chromatography

จากนั้นยืนยันสารด้วย HPLC-PDA, LC-MS/MS และ proton NMR

4. การห่อหุ้มด้วยเลซิทิน

ผสมสารสกัด trans-resveratrol กับเลซิทิน 2% ในอัตราส่วนสารสำคัญต่อสารห่อหุ้ม 1:1 และ 1:2 แล้วทำแห้งแบบแช่เยือกแข็งหรือ **lyophilization**

ประเมินประสิทธิภาพการห่อหุ้ม ขนาดอนุภาค PDI ค่า zeta potential ลักษณะพื้นผิวด้วย SEM และการปลดปล่อยในสภาวะจำลองกระเพาะอาหารและลำไส้

ผลการศึกษาที่สำคัญ

การสกัดที่เหมาะสม

สภาวะที่เหมาะสมที่สุดคือ

- เอทานอลต่อน้ำ 95:5
- อุณหภูมิ 30 องศาเซลเซียส
- ระยะเวลา 24 ชั่วโมง

ได้ trans-resveratrol ประมาณ **23.5 มิลลิกรัมต่อลิตร** วิธีสกัดด้วยตัวทำละลายแบบดั้งเดิมให้ผลดีกว่าวิธีรีฟลักซ์ อัลตราโซนิก และไฮโดรไลซ์ด้วยกรด

เมื่อเพิ่มอุณหภูมิ ปริมาณ trans-resveratrol ลดลงอย่างชัดเจน และไม่ตรวจพบที่ 70–80 องศาเซลเซียส สะท้อนว่าสารนี้ไวต่อความร้อนและอาจเปลี่ยนเป็น cis-resveratrol หรือสลายตัวได้

การทำให้บริสุทธิ์

- คอลัมน์ซิลิกาเจลช่วยกำจัดสีและสิ่งเจือปนได้บางส่วน
- Sephadex LH-20 ให้ผลดีในการกำจัดสิ่งเจือปนและได้ trans-resveratrol ที่บริสุทธิ์กว่า

- Flash chromatography ช่วยแยกสารได้ แต่บางระบบยังมีค่าความบริสุทธิ์ของพีกเพียงประมาณ 0.89 จึงยังต้องปรับสภาวะเพิ่มเติม

LC-MS/MS ตรวจพบ precursor ion ที่ m/z 227.07 และ fragment ions ที่สอดคล้องกับ trans-resveratrol ส่วน NMR แสดงสัญญาณโปรตอนที่สอดคล้องกับโครงสร้างของสาร จึงยืนยันได้ว่าสารที่แยกได้คือ trans-resveratrol

ประสิทธิภาพการห่อหุ้ม

ประสิทธิภาพการห่อหุ้มขึ้นกับปริมาณเลซิทิน

- อัตราส่วน 1:1 ห่อหุ้มได้ประมาณ **65%**
- อัตราส่วน 1:2 ห่อหุ้มได้ประมาณ **80%**

จึงแสดงว่าการเพิ่มสัดส่วนเลซิทินช่วยกักเก็บ resveratrol ได้ดีขึ้น

สมบัติของอนุภาค

- ค่า PDI ต่ำกว่า 0.5 แสดงว่าการกระจายขนาดอนุภาคอยู่ในระดับยอมรับได้
- ค่า zeta potential ของเลซิทินเปล่าประมาณ −73.4 mV
- หลังห่อหุ้ม resveratrol มีค่าประมาณ −56.6 mV

ค่าประจุที่เป็นลบสูงแสดงว่าระบบมีเสถียรภาพทางคอลลอยด์และอนุภาคไม่รวมตัวกันง่าย

ภาพ SEM พบอนุภาคค่อนข้างกลม มีพื้นผิวขรุขระ และไม่พบผลึก resveratrol ชัดเจนบนผิว สนับสนุนว่าสารส่วนใหญ่ถูกกักเก็บอยู่ภายในโครงสร้างเลซิทิน

การปลดปล่อยในระบบทางเดินอาหารจำลอง

ในสภาวะกระเพาะอาหาร pH 1.5

- resveratrol อิสระแทบไม่ละลาย
- resveratrol ที่ห่อหุ้มมีการปลดปล่อยเพิ่มขึ้นอย่างช้า ๆ จนถึงประมาณ 25%
- แสดงว่าสารยังคงค่อนข้างเสถียรในกระเพาะอาหาร

ในสภาวะลำไส้ pH 6.8

- สูตรที่ห่อหุ้มละลายและปลดปล่อยสารได้ดีกว่ามาก
- การปลดปล่อยสูงถึงประมาณ **80% ภายใน 2 ชั่วโมง**
- จึงมีแนวโน้มเพิ่มโอกาสที่ resveratrol จะถูกดูดซึมจากลำไส้

ผลนี้แสดงว่าการห่อหุ้มด้วยเลซิทินทำให้เกิดการปลดปล่อยที่ขึ้นกับค่า pH โดยช่วยปกป้องสารในกระเพาะอาหารและปลดปล่อยมากขึ้นเมื่อเข้าสู่ลำไส้

ความสำคัญของงานวิจัย

งานวิจัยนี้แสดงแนวทางการเพิ่มมูลค่าให้กากองุ่นจากของเสียอุตสาหกรรม โดยเปลี่ยนให้เป็นแหล่งของสารออกฤทธิ์ทางชีวภาพ สามารถเชื่อมโยงกับแนวคิดเศรษฐกิจหมุนเวียนและ BCG Economy ได้

ระบบเลซิทิน–resveratrol มีศักยภาพสำหรับพัฒนาเป็น

- อาหารฟังก์ชัน
- ผลิตภัณฑ์เสริมอาหาร
- ส่วนประกอบในผลิตภัณฑ์ยา
- ระบบนำส่งสารต้านอนุมูลอิสระ

ข้อจำกัด

ผลการทดลองเป็นเพียงการศึกษาการปลดปล่อย **in vitro** จึงยังไม่ยืนยันว่าชีวปริมาณออกฤทธิ์ในมนุษย์เพิ่มขึ้นจริง นอกจากนี้ยังขาด

- การทดลองในสัตว์หรือมนุษย์
- การศึกษาการดูดซึมและเภสัชจลนศาสตร์
- การประเมินความเป็นพิษ
- การศึกษาความเสถียรระหว่างการเก็บรักษาระยะยาว
- การเปรียบเทียบกับสารห่อหุ้มชนิดอื่น

บทความยังรายงานขนาดอนุภาคบางส่วนว่าเล็กกว่า 157–160 นาโนเมตร แต่ในผล DLS อีกส่วนรายงานค่า Z-average ประมาณ 360–442 นาโนเมตร จึงควรตีความเรื่องขนาดอนุภาคอย่างระมัดระวังและต้องมีการชี้แจงวิธีคำนวณหรือชนิดของค่าขนาดที่รายงาน

สรุปสาระสำคัญ

กากองุ่นสามารถใช้เป็นแหล่งสกัด trans-resveratrol ได้ โดยควรสกัดที่อุณหภูมิต่ำด้วยเอทานอล–น้ำ การห่อหุ้มด้วยเลซิทินโดยเฉพาะอัตราส่วน 1:2 ให้ประสิทธิภาพสูงประมาณ 80% ช่วยเพิ่มการละลาย ความเสถียร และการปลดปล่อยในสภาวะลำไส้ แต่ยังต้องมีการทดลองในสิ่งมีชีวิตก่อนสรุปว่าเพิ่มชีวปริมาณออกฤทธิ์และประสิทธิผลทางสุขภาพได้จริง

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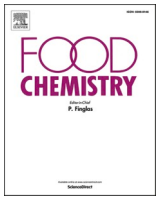

Extraction, purification of bioactive *trans*-resveratrol from grape pomace as factory waste, and enhancement of bioavailability through encapsulation technology

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ABSTRACT

Trans-Resveratrol is a key phenolic compound with known health benefits but suffers from low bioavailability and instability. Its encapsulation is challenging due to its polarity. In this study, *trans*-resveratrol was isolated, purified, and structurally characterized from grape pomace, a wine industry by-product, using LC-MS/MS, HPLC-PDA, and ^{1}H NMR. To improve its stability and solubility, nanoencapsulation was carried out with lecithin via lyophilization. The resulting nanoparticles had sizes below 160 nm, a PDI under 0.5, and high colloidal stability as shown by zeta potential analysis. In vitro release tests in simulated gastric and intestinal fluids revealed enhanced release for the lecithin-encapsulated formulation, achieving up to 80 % in intestinal conditions, unlike free *trans*-resveratrol. These findings suggest that grape pomace is a valuable source of bioactive compounds and that lecithin-based nanoencapsulation offers a promising strategy to enhance the delivery and bioavailability of trans-resveratrol.

1. Introduction

The rapid growth of the world's population and the impact of industrialization have made the management and utilization of factory waste one of today's most critical environmental and resource management issues. Therefore, the effectiveness of waste management and recycling processes has become increasingly important. Factory waste, especially in the food industry, can lead to various environmental problems and result in the loss of valuable resources. Numerous studies have been conducted to eliminate these issues and to evaluate these valuable components. Food waste contains components with high nutritional value, and their loss leads to both economic and environmental negative outcomes. Proper processing and utilization of these wastes have become inevitable in this regard. Additionally, in recent years, there has been increasing interest in phenolic compounds derived from fruits due to their bioactive roles. Grapes are among the most widely cultivated fruits in the world (Özdüven et al., 2005). Grapes are processed in wineries and juice factories, resulting in grape pomace as a by-product. Grape pomace, consisting mainly of skin and seeds, contains highly valuable components, particularly resveratrol. However, its improper disposal contributes to environmental pollution. The increase in environmental pollution has become a significant global issue threatening ecosystems and human health (Monier et al., 2010). To prevent environmental pollution and effectively utilize waste, various strategies have been developed and implemented.

Grapes, a product of the Vitaceae family and Vitis genus, are known for their greenish-yellow or purple-black fruits, and are cultivated in various forms such as vines and shrubs. Grapes are among the most widely grown fruits globally, with over 7 million hectares dedicated to cultivation (Alston & Sambucci, 2019). There are over 15,000 varieties of grapes, and in Turkey, table and wine grapes are cultivated in almost every region (Sümbül, 2022, Sevindik et al., 2017). Approximately 71 % of grape production is used for wine, 27 % for table grapes, and 2 % for dried fruits (Keskin et al., 2017; *TİM - Türkiye İhracatçılar Meclisi*, 2025). In this context, the amount of grape pomace, discarded as waste, is significant. Processed grape pomace typically consists of 50 % skins, 25 % seeds, and 25 % stems (Teixeira et al., 2014). If not properly utilized, this waste can contribute to significant environmental pollution. Grape pomace, rich in polyphenols, contains highly valuable compounds. Generally, it consists of anthocyanins, catechins, flavanol glycosides, phenolic acids, and alcohols, with stilbenes as the primary components. Research has focused on examining the polyphenol content in grape

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seeds, skins, and stems. Red grape skin extract, for example, is richer in phenolic content and resveratrol than the seeds (Negro et al., 2003). Similar findings have been observed when comparing white grapes (Martín-Carrón et al., 2000). Additionally, grape skin extracts have shown higher antioxidant activity compared to other parts (Shaker, 2006). The health benefits of these polyphenolic compounds have been well-established through numerous studies (Keskin et al., 2009). In recent years, especially due to the impact of the pandemic, there has been an increased interest in polyphenolic compounds derived from natural sources. Many studies have demonstrated their therapeutic potential for various diseases, highlighting fewer side effects compared to conventional chemical drugs. Polyphenolic compounds, the largest family within the plant kingdom, host a variety of beneficial compounds, including *trans*-resveratrol.

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a phenolic compound with the chemical formula $C_{14}H_{12}O_3$ (Wenzel et al., 2005). It is naturally found in many plants, with a high concentration in red grapes. Particularly abundant in grape skins, resveratrol is a secondary metabolite synthesized for defensive purposes, protecting the plant against fungal infections. This molecule contains a stilbene core, comprising two phenyl rings and four carbon-carbon double bonds (C=C). On each side of the stilbene core, hydroxyl (-OH) groups are present. Resveratrol has low water solubility, with *trans* and *cis* isomers existing. *Trans*-Resveratrol contains more bioactive compounds than *cis*-resveratrol. Being sensitive to UV light, heat, and light, *trans*-resveratrol undergoes isomerization to cis-isomer upon exposure (Fig. 1).

The health benefits of resveratrol, a stilbene compound, have been demonstrated through numerous studies (Creasy and Creasy, 1998) (See Fig. 2).

The potential health benefits of resveratrol were highlighted in a study published by French scientists in 1992. In this research, it was suggested that resveratrol could have a protective effect against cardiovascular diseases (Siemann and Creasy, 1992, Soleas, Goldberg, et al., 1997). A significant study demonstrated that *trans*-resveratrol inhibits LDL oxidation, reducing the risk of atherosclerosis. Additionally, other studies have shown that *trans*-resveratrol can lower blood pressure and prevent blood clotting, supporting its role as a critical compound in cardiovascular health (Liu et al., 2017).

The cardioprotective effects of resveratrol are primarily associated with the suppression of oxidative stress, the reduction of inflammatory processes, and its beneficial impact on various cardiovascular risk factors (Gal et al., 2021). In a recent experimental study conducted in 2020, resveratrol administered at a dose of 20 mg/kg for 10 weeks was reported to have beneficial effects on cholesterol levels in diabetic rats (Szkudelska et al., 2020). These findings support the consideration of trans-resveratrol as a critical compound in the maintenance of cardiovascular health. Some studies have shown that resveratrol administration leads to an increase in serum and bone alkaline phosphatase levels; however, it does not cause significant changes in serum calcium, osteocalcin (a specific biomarker indicating bone turnover), or procollagen levels.

In a clinical study conducted on individuals with type 2 diabetes and at risk of fractures, a daily supplementation of 500 mg resveratrol resulted in a smaller decrease in bone mineral density (Bo et al., 2018; Scarano et al., 2019). The antiviral effect of resveratrol has been reported to be associated with the activation of SIRT-1 pathways, which enhance cellular resistance against viruses (Côté et al., 2015). Studies conducted on COVID-19 have revealed that resveratrol has the potential to inhibit the entry and replication of the virus in host cells(Inchingolo et al., 2021a; Inchingolo et al., 2021b; Yang et al., 2020). Numerous experimental studies have demonstrated that resveratrol exhibits immunomodulatory effects on the immune system and supports immune functions. Resveratrol modulates both innate and adaptive immune responses by interacting with various cellular targets (Alesci et al., 2022; Ávila-Gálvez et al., 2021). In recent years, research has indicated that resveratrol slows and even kills the growth of cancer cells (Ren et al., 2021).

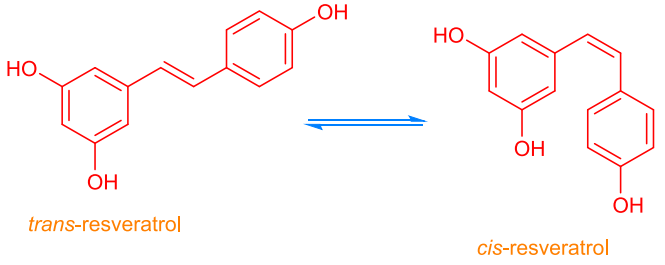

Fig. 1. *Trans* and *cis* resveratrol isomerization.

Some studies suggest that resveratrol may have potential in preventing certain types of cancer, such as prostate, breast, and colon cancer (Chen et al., 2006). As an antioxidant polyphenol, resveratrol exhibits a wide spectrum of effects in the fields of medicine and pharmaceuticals. Understanding the positive effects of resveratrol has led to increased production of herbal extracts and dietary supplements containing this valuable compound. Although resveratrol has many beneficial properties, it also has some disadvantages. These include its unstable structure, sensitivity to oxidation, and degradation due to environmental factors such as heat and light. Additionally, it has low bioavailability, which affects its stability and effectiveness, limiting its industrial and pharmaceutical applications and usage. To overcome these limitations and increase its bioavailability, various mechanisms have been developed. Several studies have shown that resveratrol, when taken orally, is not sufficiently absorbed by the digestive system and is rapidly metabolized by the liver. Previous research revealed that resveratrol, used solely as a pure substance (*trans*-resveratrol), has low bioavailability values (Wenzel and Somoza, 2005).

Resveratrol is a bioactive compound with limited bioavailability due to its low water solubility, susceptibility to photodegradation, and rapid metabolism. Therefore, in recent years, various delivery systems have been developed to enhance the stability, absorption, and overall bioavailability of resveratrol. Notably, significant progress has been made in this field over the last decade (Dikmetas et al., 2024). Among the most commonly employed encapsulation strategies for improving resveratrol delivery are spray-drying techniques (Consoli et al.), incorporation into liposomal structures (Gu et al., 2023), emulsion-based delivery systems (Fan et al., 2023), and nanocapsule technologies (Aghaz et al., 2021).

In studies focusing on spray-drying, Consoli et al., 2019) encapsulated resveratrol using Maillard conjugates and sunflower oil-based emulsions, demonstrating that Maillard conjugates provided high retention rates (80–100 %) and encapsulation efficiencies of up to 97 %. Subsequent studies by the same group (Consoli et al., 2020, 2023) compared different emulsification techniques, identifying the batch membrane emulsification method as the most effective in terms of both retention and encapsulation efficiency (97 %).

Cardoso et al. (2019) investigated systems incorporating gum arabic as the wall material, characterizing the resulting microparticles and evaluating their controlled release behavior using mathematical models such as Weibull and Korsmeyer-Peppas. The highest encapsulation efficiency recorded in this study was 87 %.

In our study, we utilized liposomes, which are known to be biocompatible due to their structural similarity to cell membranes, and are capable of encapsulating both hydrophilic and lipophilic compounds while exhibiting low toxicity (Alrashidi et al., 2024). Research has shown that resveratrol-loaded liposomes can enhance cell proliferation, protect cells from UV-induced damage, and improve cellular stress responses (Płachta et al., 2024). Furthermore, liposomal formulations have been reported to significantly increase the uptake of resveratrol by hepatocellular carcinoma cells, thereby improving its therapeutic efficacy in liver cancer treatment (Jagwani et al., 2020).

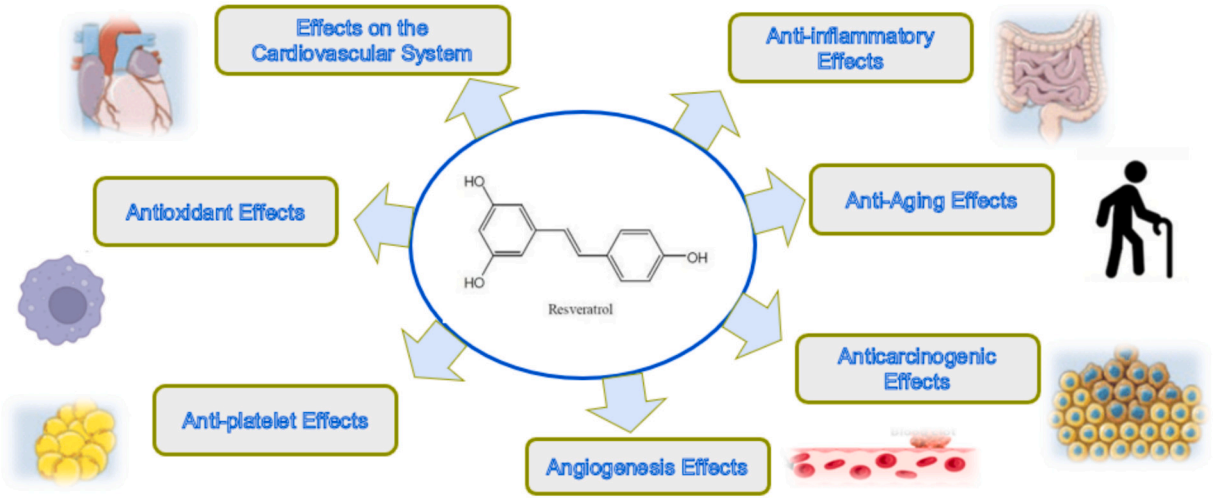

Fig. 2. Health effects of *trans*-resveratrol.

In a recent study on the development of resveratrol-loaded nanoliposomes, Liu et al. (2023) employed a combination of thin-film hydration and ultrasonication techniques, achieving structurally stable nanoliposomes with a high encapsulation efficiency of approximately 86.78 %. These nanoliposomes were comprehensively characterized in terms of in vitro release profile, antioxidant capacity, and cytotoxic effects. Compared to free resveratrol, the encapsulated form exhibited enhanced antioxidant activity and reduced cytotoxicity. The high encapsulation efficiency, controlled release profile, and biocompatible nature of these resveratrol-loaded nanoliposomes suggest that the developed delivery system holds promising potential for applications in both the food and pharmaceutical industries (Dikmetas et al., 2024).

In studies on bioavailability, Walle et al. examined the absorption and bioavailability of orally administered resveratrol. The results showed that the absorption rate of oral resveratrol was 75 %, but its bioavailability rate was less than 1 %, suggesting that higher doses might be necessary to achieve effective results, and alternative application methods should be explored (Walle, 2011). Another study investigated the effect of glycyrrhizic acid on resveratrol's oral bioavailability, finding that glycyrrhizic acid significantly increased resveratrol's bioavailability. Most studies on resveratrol bioavailability have explored whether bioavailability can be enhanced through different drug forms, formulations, or encapsulation methods. In our study, lecithin was used as an encapsulation agent due to its structural similarity to cell membranes and its natural presence in various sources. Lecithin, also known as phosphatidylcholine, typically consists of a mixture of glycolipids, triglycerides, and phospholipids. Lecithin is a phospholipid compound composed of a glycerol molecule, two fatty acids, a phosphate group, and a choline group. It naturally occurs in egg yolks, soybeans, sunflower seeds, and some other plants and is also used in processed foods and supplements. Lecithin plays a significant role as an encapsulation agent because of its emulsifying properties, making it effective in various formulations. Lecithin serves as an important component in encapsulation applications, providing oxidation stability, emulsion capacity, soft capsules, bioavailability enhancement, and various uses in food supplements.

This study was designed to investigate the purification of resveratrol from grape pomace, a factory waste, and the encapsulation of purified resveratrol with lecithin. The goal is to prevent the high-value product, resveratrol, from being wasted as a byproduct of wine and juice factories, promoting its value-added utilization. Additionally, encapsulation of resveratrol with lecithin aims to improve its stability and increase its bioavailability, which is typically low due to its instability and low solubility in water. In this context, the extraction and purification steps of resveratrol from grape pomace obtained from wineries and juice factories were optimized using a newly developed HPLC-PDA (High-performance Liquid Chromatography) procedure. Structural analysis of the obtained *trans*-resveratrol was carried out using LC-MS/MS, HPLC-PDA, and H-NMR analyses. Following encapsulation, in vitro gastrointestinal release tests were conducted, with controlled release behavior assessed using high-performance liquid chromatography with Photodiode Array Detector (HPLC-PDA). These controlled release profiles helped explain the behavior of resveratrol encapsulated with lecithin, focusing on improving its stability and bioavailability.

2. Materials and methods

2.1. Plant materials

For the study, grape pomace was collected through collaboration with wineries. Research was conducted using three types of grape pomace from wineries in Kırklareli, Manisa, and Ankara regions. The collection of waste products began in August 2020 through the winery. The pomace was processed to remove impurities, discard spoiled skins, and clean using water to eliminate dust, achieving a moisture level of $\leq$5 %. The processed pomace was then ground into a powder with a particle size of 40–60 mesh using a Vita-prep™ blender.

2.2. Chemicals and devices

LC-MS/MS8030 Plus, H-NMR JEOL ECZ500R (11.75 Tesla), FEI Quanta FEG 250 SEM, Ultrapure Water (Thermo Scientific), Ultrasonic Bath (İntersonik min-12), HPLC-PDA (Shimadzu LC20), Magnetic Stirrer (Termal), $\geq$99.8 % Ethanol (Sigma Aldirch, HPLC Grade),C_{18} Column (250mmx4,6X5µm, NANOLOGİCA SVEA), 85 % ortho-phosphoric acid (ISOLAB),$\geq$99.9 % Acetonitrile (ISOLAB),$\geq$99.9 % Methanol (ISOLAB), Ethyl alcohol (Merck 100,983), Formic acid (Sigma Aldrich 251,364), *trans*-Resveratrol $\geq$99.9 % (Merck 501–36-0), Lecithin (Alfasol), Hydrochloric acid (Merck 100,317), Sodium hydroxide (106498), Ethyl Acetate (Merck 100,868), NaCl (Merck 106,404). In this study, all reagents were of analytical quality, and ultra-pure water was used for the preparation of all solutions.

2.3. Extraction processes

2.3.1. Classical solvent extraction

The classical solvent extraction method is a useful and standardized approach for *trans*-resveratrol extraction (Crăciun & Gutt, 2023). In classical solvent extraction, independent variables such as solvent type (ethanol, methanol, distilled water, MeOH-NH_4OH) (Creasy & Coffee,

1988) and concentration (50 %, 80 %, 100 %), temperature (30, 60, 70, 80 °C), and extraction time (30 min, 1 h, 3 h, 24 h) (Pezet et al., 2003) were determined. During the extraction method determination, experiments listed in Table 1 were performed. Ten grams of powdered grape pomace were extracted with 100 mL of solvent using a magnetic stirrer. The extract was filtered and evaporated to dryness in a rotary evaporator at room temperature. Dissolved in ethanol, filtered through a 0.45 μm PTFE filter, and stored in vials. Until analysis, it was stored at −20 °C. Extraction yield was calculated by dividing the mass of the recovered dry extract (mr) by the initial mass of the grape pomace powder (mi) (Eq. (1)), and the resveratrol content was determined using HPLC-PDA.

$$\%Yield = mi \div mr \times 100 \quad (1)$$

2.3.2. Acidic medium extraction

Acidic medium extraction was applied before ethanol extraction to remove unwanted anthocyanin molecules from the solution. Ten grams of powdered grape pomace were mixed with 100 mL of distilled water containing 1 % hydrochloric acid at 25 °C for 3 h. Then, the mixture was filtered using Whatman filter paper to separate the solution, and the solid residue was extracted with 100 mL of ethanol. The extract was filtered and evaporated to dryness in a rotary evaporator. The solid residue was dissolved in ethanol, filtered through a 0.45 μm PTFE filter, and stored in vials. Until analysis, it was stored at −20 °C. Extraction yields were calculated using Eq. (1), and resveratrol content was determined using HPLC-PDA.

2.3.3. Reflux ethanol extraction

10 g of powdered grape pomace were precisely weighed and transferred into a flask with 100 mL of ethanol solvent. Extraction was carried out under a reflux condenser for 2 h (Huiying and Zhan, 2016). Afterward, filtration was performed to separate the solid residue. The obtained extract was evaporated to dryness using a rotary evaporator, and the solid residue was dissolved in ethanol, filtered through a 0.45 μm PTFE filter, and stored in vials. Until analysis, it was stored at −20 °C. Extraction yields were calculated using Eq. (1), and resveratrol content was determined using HPLC-PDA.

2.3.4. Ultrasonic assisted extraction

A solvent extraction system supported by ultrasonication was designed to operate at both room temperature and 60 °C. A solvent mixture of 80:20 (*V*/V) ethanol/water solution was used, with a duration of 30 min and a sample-to-solvent ratio of 1/10 (Romero-Pérez et al., 2000; Yong-Jin, 2006). After extraction, filtration was performed to separate the solid residue, and the obtained extract was evaporated to dryness. The remaining solid was dissolved in ethanol, filtered through a 0.45 μm PTFE filter, and placed into vials. Extraction yields were calculated using Eq. (1), and resveratrol content was determined using HPLC-PDA.

Table 1
Extraction experiments.

Grape Pulp (Gram)	Extraction Solvent (V/V)	Extraction Time (min)	Extraction Temperature (°c)	Liquid /solid ratio (valume/ mass, mL/g)
10	Ethanol	180	30	1:5; 1:10; 1:15; 1:20
10	Ethanol 50 %	180	30,70	1:10
10	Ethanol 80 %	180	30	1:10
10	Ethanol 80 %	30 min	60	1:10
10	Ethanol 95 %	1	70	1:10
10	Ethanol 100 %	1	80	1:10
10	Methanol 80 %	1	30	1:10
10	pure water 100 %	12	30	1:10
10	99,5 / 0,5 (Methanol-Ammonium hydroxide)	24	30	1:10

2.3.5. Acid hydrolysis

According to the method reported in the literature, 1/10 (g/mL) solid/liquid ratio of ground grape pomace was extracted with 95 % ethanol at 50 °C for 20 min (Al-Jubouri et al., 2022). After extraction, the pH of the supernatant was adjusted to 1 using hydrochloric acid, and then heated under reflux at 57 °C for 8 h. This process facilitates the hydrolysis of resveratrol dimers, trimers, and glycosides. Following hydrolysis, a liquid-liquid extraction was performed using a mixture of ethyl acetate and ether, and the pH was adjusted to 7 with sodium carbonate. The solid residue was separated by filtration, and the obtained extract was evaporated to dryness. The remaining solid was dissolved in 10 mL ethanol, filtered through a 0.45 μm PTFE filter, and placed into vials. Resveratrol content was stored at 20 °C until analysis.

2.3.6. Liquid – liquid extraction

Ground grape pomace was first mixed with a solvent at a 1/10 (g/mL) solid/liquid ratio at 25 °C for 3 h. The solid residue was separated by filtration. To remove unwanted impurities, liquid-liquid extraction was performed using organic solvents (Galeano et al., 2007). Initially, after filtration, 5 mL of distilled water and 35 mL of hexane were added to the remaining liquid and extracted. The hexane phase was separated in a separating funnel. This process was repeated two more times. The hexane phases were combined, and the ethanol-water mixture phase was evaporated until concentrated. In the second step, 25 mL of ethyl acetate and 25 mL of distilled water were added and separated in a separating funnel. The ethyl acetate phase was then evaporated to dryness and vialed for storage and analysis.

2.4. Purification processes

2.4.1. Silica gel column chromatography

To obtain pure resveratrol, the residue was dissolved in ethanol (Nakanishi et al., 1998). The optimal solvent system for the column was determined using TLC. Before loading onto the column, the mobile phase (chloroform, ethyl acetate, and formic acid in a 25:10:1 (v:v:v) ratio) was re-dissolved and applied to a pre-conditioned silica gel column (2.5 × 21 cm) with the same composition. The column was eluted using a mixture of 25:10:1 chloroform, ethyl acetate, and formic acid. Eluents were collected in 25 fractions of 15 mL each, and TLC analysis was performed on fractions showing positive results. The positive fractions were combined and dried under a rotary evaporator. TLC analysis was conducted on silica gel 60 F_{254} aluminum plates, and both standard and corresponding extract spots exhibited clear purple fluorescence when exposed to UV light at 254 nm. The fractions were dissolved in 2 mL of ethanol and stored for HPLC-PDA analysis.

2.4.2. Sephadex LH-20 column chromatography

Post-extraction purification was carried out using an open glass column (2.5 × 21 cm) filled with Sephadex LH-20. The residue was dissolved in 100 μL of ethanol and re-dissolved in a mobile phase composed of hexane:ethyl acetate (1, 9) before being loaded onto the column. Elutions were collected in 10 fractions of 1 mL each. TLC analysis identified positive resveratrol spots in the fractions, which were combined and dried under a rotary evaporator. TLC was performed on silica gel 60 F_{254} aluminum plates, and both standard and corresponding extract spots showed clear purple fluorescence under UV light at 254 nm. The fractions were dissolved in 2 mL of ethanol and stored for HPLC-PDA analysis.

2.4.3. Flash chromatography

The purification of *trans*-resveratrol extracted using an optimized method was carried out using two different columns: SiliaSep™ C_{18} (12

g) and SiliaSep™ HP (12 g). The columns were equilibrated with a gradient system before separation. Different solvent systems and flow rates were applied in gradient systems for the separation of *trans*-resveratrol (Table 2).

The separated fractions were collected and monitored using ELSD (Evaporative Light Scattering Detector), as well as at wavelengths of 254, 265, 280, and 305 nm. The collected fractions were dried using a rotary evaporator and analyzed by HPLC-PDA.

2.4.4. High-performance liquid chromatography-electrospray photo diode Array mass spectrometry (HPLC-PDA)

In this study, qualitative and quantitative determinations of *trans*-resveratrol content at various stages extraction, purification, and release tests were conducted using a new HPLC-PDA method. HPLC-PDA analyses were performed using a Shimadzu LC-20AT model device. The gradient elution program used in the HPLC-PDA analysis is detailed in Table 3.

Column Temperature: 30 °C, Column: C_{18} Column (250 mm × 4.6 × 5 µm, NANOLOGICA SVEA), Flow Rate: 0.8 mL/min, Injection Volume: 20 µL, Detector: PDA, *Trans*-resveratrol was identified by comparing its retention time with the corresponding peak in the standard compound and its UV spectrum. The concentrations of *trans*-resveratrol were measured using an external standard method, utilizing calibration curves obtained for this compound within the observed concentration range. Quantitative data were presented in mg/L (ppm) per gram of sample.

2.4.5. Validation of the Present Method—Quantification of Constituents

In this study, a calibration curve was created for the quantification of *trans*-resveratrol using HPLC-PDA analysis. The *trans*-resveratrol standard was obtained from Sigma Aldrich. Initially, standard solutions were prepared at concentrations of 0.625, 1.25, 2.50, 5.00, 7.50, and 10.00 µg/mL, each concentration analyzed in duplicate. The area values obtained from these solutions were used to plot the calibration curve, and the method's linearity was calculated based on the study conducted by Karagul et al. (2024). The applicability of the method was determined through this linearity assessment.

Linearity was determined by calculating the correlation coefficient ($R^2 = 0.99$) within the range of 0.625–10.00 µg/L. The calibration curve of resveratrol is presented in Fig. S3 (Supporting Information). Analytical method validation characteristics were as follows: Linearity Equation: y = 110,800× – 18,361, LOD (µg/mL): 0.33, LOQ (µg/mL): 0.99, Recovery (%): 100.84

2.4.6. LC–MS/MS and 1H NMR spectrometry analysis

The purified final product's main component was confirmed to be *trans*-resveratrol through preparative HPLC-PDA, and its chemical structure was further characterized using LC-MS/MS and ^{1}H NMR. Mass spectrometry was performed using the Thermo TSQ Quantis LC-MS/MS system for qualitative analysis. The precursor molecular ion of *trans*-resveratrol, with a molecular formula of $C_{14}H_{12}O_3$ and a molecular weight of 228,25 g/mol, was detected at m/z = 228,250. When the precursor ion was sent through the second quadrupole with a collision energy of 19 V, it was fragmented, and the mass/charge (m/z) ratios were separated, revealing characteristic fragmentation ions. ^{1}H NMR analysis was carried out using the JEOL ECZ500R (11.75 Tesla) NMR device. The purified *trans*-resveratrol compound was prepared in methanol–d_6 (CD_3OD–d_6), and the chemical shift values for protons in different chemical environments were determined from the NMR spectrum.

Table 2
Applied methods.

A Mobile Phase	B Mobile Phase	Cartridge (12 g)	Flow rate (mL/min)	%B change	Time (min)
Hexane	Ethyl Acetate	C_{18} & Si	10, 20, 30, 50	%0–100	30
Water	Methanol	C_{18} & Si	10, 20, 30, 50	%0–100	30

Table 3
Gradient elution programme.

Time	%A (ACETIC ACID/ACN/WATER (v/v) 0,5 / 149,5 / 350 mL)	%B (% 99.9 Acetonitrile)
0	75	25
20	25	75
23	75	25
30	75	25
31	Equilibration	

2.5. Production of powder microcapsules using lyophilization method

The encapsulation of *trans*-resveratrol using lecithin as the coating material was performed using the lyophilization method. This process aimed to develop a *trans*-resveratrol drug delivery system with increased solubility and bioavailability. In the first step, 2 % lecithin powder was dissolved in distilled water and stirred overnight at room temperature using a magnetic stirrer. Subsequently, the enriched *trans*-resveratrol solution was mixed, creating two different ratios (1,1 and 1,2 core to coating material ratio). The homogenized mixture was prepared by intensive stirring for 24 h before undergoing lyophilization (freeze-drying) (Fig. 3).

To prepare freeze-dried lecithin-loaded RES powder, wet microcapsules were first frozen for 24 h at −20 °C, followed by drying in a lyophilizer (BK-FD12P, Biobased) at −85 °C for 72 h while maintaining a vacuum pressure of 1 Pa. The resulting powder was collected and stored in a desiccator for SEM analysis. The capsules produced were named Lecithin-RES capsules.

2.6. Encapsulation efficiency

The encapsulation efficiency of Lecithin-RES samples was evaluated by measuring the RES content. The powder microcapsules were incubated in a release medium (ethanol). The resulting solution was filtered, and the free active substance was quantified using HPLC-PDA analysis. Encapsulation efficiency (EE) was calculated using the following equation (Ren et al., 2019).

$$\text{EncapsulationEfficiency}(\%) = \frac{BinitialRSVamount - FreeRSVAmount}{InitialRSVAmount} \text{x}100 \quad (1)$$

2.7. Zeta potential and particle size analysis

The zeta potential and particle size analyses of the nanoparticle formulations were performed using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, UK). Prior to measurement, the samples were diluted with deionized water at a ratio of 1:10 and gently vortexed. All measurements were conducted at 25 ± 0,5 °C using clear disposable zeta cells. Ultrapure water (refractive index: 1330; viscosity: 0,8872 cP; dielectric constant: 78.5) was used as the dispersant.

Zeta potential values were calculated based on electrophoretic mobility using the Smoluchowski approximation. Particle sizes were expressed as intensity-weighted hydrodynamic diameters (*Z*-Average), and the polydispersity index (PDI) was employed to assess the uniformity of the particle size distribution. Each sample was measured in triplicate, and mean values with standard deviations were reported. The formulations were designated as L-1 (lecithin only) and L-2 (lecithin loaded with resveratrol).

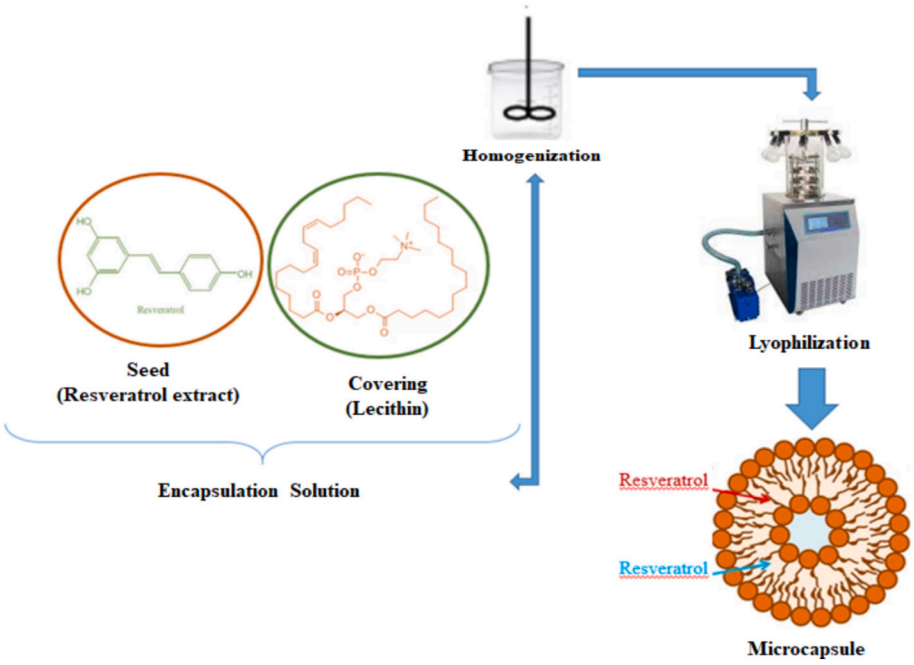

Fig. 3. Lyophilization method.

2.8. Image analysis by scanning Electron microscope (SEM)

The surface morphology of Lecithin-RES microcapsules was analyzed using SEM (FEI Quanta FEG 250 Model) at 20 kV increasing voltage under high vacuum. Images were captured at magnifications of 500×, 1000×, and 5000× for analysis.

2.9. In vitro release studies

In vitro release studies of Lecithin-RES microcapsules were conducted in HCl (pH 1.5, gastric pH), KCl buffer (pH 6.8, intestinal pH) at 37 °C. For this purpose, RES-loaded lecithin capsules (15 mg) were suspended in 30 mL of solution environments at pH 1.5 and pH 6.8, respectively, in closed containers. Every 30 min, 2 mL of solution was collected for HPLC-PDA analysis. An equal volume of buffer solution was added back to maintain the system's volume. These steps were continued until the end of 2 h, and the collected solutions were vialed for HPLC-PDA analysis. *Trans*-Resveratrol without encapsulation served as the control group (Merve, 2018; Li et al., 2023).

The release values of resveratrol from Lecithin-RES capsules were calculated according to the following equation.

$$\%\textbf{Release} = \text{Mt.}/\text{Mo} \times 100$$

Mt. = The amount of resveratrol released at time t is.
Mo = Initial amount of resveratrol loaded ($t = 0$).

2.10. Statistical analysis

All experimental studies were conducted with at least three independent replicates ($n = 3$). The obtained data are presented as mean ± standard deviation (SD). Observed differences among measurements were evaluated, and a significance level of $p < 0.05$ was considered statistically significant. The results were interpreted by taking into account only the differences that were consistently observed across replicates and found to be statistically meaningful.

3. Results and discussion

3.1. Validation results

The *trans*-resveratrol standard (≥99 % (HPLC), Sigma Aldrich) was prepared in methanol solvent (99.5 %, HPLC grade, ISOLAB) at a concentration of 10 ppm. The calibration curve exhibited excellent linearity with $R^2 = 0.999$ and a %RSD of 3.36. The detection wavelength for *trans*-resveratrol was identified as 305 ± 1 nm. Fig. 4 includes a standard HPLC-PDA chromatogram with: a) UV spectrum scan and specific wavelength related to the standard, b) visual representation including peak purity.

The system suitability parameters for the HPLC-PDA analysis of resveratrol were evaluated and are presented in Table 4.

3.2. Optimization of Extraction Conditions with HPLC-PDA

In optimizing extraction parameters, variables included potential solvent (A), temperature (B), and extraction time (C). The contents of *trans*-resveratrol in the extracts are presented in the following fig. 5.

The results indicated that increasing the extraction temperature led to a significant decrease in *trans*-resveratrol yield. Notably, at 70 °C and 80 °C, *trans*-resveratrol could not be detected by HPLC-PDA. This finding is consistent with previous studies reporting that *trans*-resveratrol is thermally unstable and tends to isomerize into its *cis* form under elevated temperatures (Soleas et al., 2001; Gambini et al., 2015). As a phenolic compound, *trans*-resveratrol is highly sensitive to environmental conditions such as heat, light, and oxygen.

On the other hand, an increase in extraction time resulted in a gradual rise in *trans*-resveratrol yield. The maximum yield was achieved at 24 h (1440 min), while no significant increase was observed at 36 h (2160 min), suggesting that the extraction process reached a plateau. This plateau effect may be attributed to the establishment of thermodynamic equilibrium over prolonged extraction periods (Vergara-Salinas et al., 2012).

Based on these findings, the optimal extraction conditions for maximizing trans-resveratrol yield were determined as follows: solvent ratio of ethanol:water (95:5, v:v), extraction temperature of 30 °C, and extraction time of 24 h. These parameters allow for the highest recovery

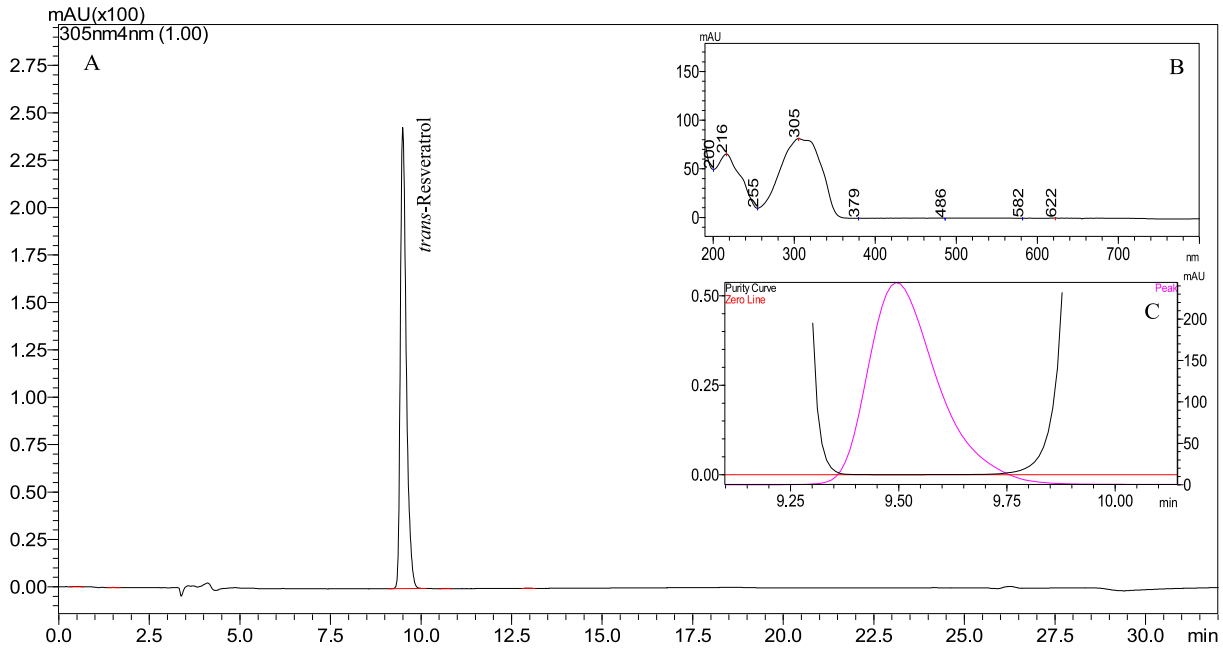

Fig. 4. HPLC chromatogram. *Trans*-Resveratrol (A), UV spectrum scan and specific wavelength (B), peak purity of *trans*-resveratrol (C).

Table 4
System Suitability Parameters for Resveratrol.

Compound Resveratrol	Retention Time	Tailing Factor (avg 1077)	k' (avg. 2.187)
%RSD	0,074	0,009	0,426
Std. Dev	0,005	0,001	0,009

Std.Dev = Standard Deviation.
%RSD = Relative Standard Deviation.
K′: Resolution.

of the bioactive *trans*-resveratrol form while minimizing degradation or isomerization.

3.3. Comparison of resveratrol levels of different extraction methods by HPLC-PDA

In selecting the extraction method, support was drawn from literature data. Numerous extraction methods have been suggested in the literature (Wang et al., 2013). The extraction methods compared include classical solvent extraction, acidic environment extraction, reflux, ultrasonic-assisted extraction, acid hydrolysis, and liquid-liquid extraction methods. In our study, it was found that the traditional solvent method yielded higher results compared to other methods. The optimized classical solvent extraction method, using Ethanol/Water (95:5) at an extraction temperature of 30 °C and 24 h of extraction, yielded a *trans*-resveratrol concentration of 23,5 mg/L. However, the purity of the peak was found to be 0.88, indicating the presence of other molecules that could contribute to impurities along with *trans*-resveratrol. Experimental results are presented in the Fig. 6.

A bar graph showing the yield of *trans*-resveratrol for each extraction method. The highest yield was obtained using the traditional solvent extraction method, utilizing ethanol at 30 °C for 24 h. The other methods tested showed very low extraction yields. The low levels of *trans*-resveratrol are attributed to the molecule's sensitivity to factors such as heat, light, and temperature, leading to degradation or conversion into the *cis* form. Therefore, extraction methods like reflux and ultrasonic bath extraction may contribute to the conversion to the *cis* form, reducing the overall *trans*-resveratrol levels and potentially increasing the formation of *cis*-resveratrol. In a reported study, UV exposure has been shown to lead to the conversion from *trans* to *cis* form (Nour et al., 2012). HPLC-PDA chromatograms indicate different retention times for *trans*-resveratrol and *cis*-resveratrol, making it likely that lower *trans*-resveratrol levels could be observed. Additionally, other extraction methods such as acidic environment extraction, acid hydrolysis, and liquid-liquid extraction also yielded low *trans*-resveratrol levels and were deemed unsuitable for the extraction of *trans*-resveratrol from grape pomace.

3.4. Determination of purification method by HPLC-PDA

The preference for silica filler due to its polar nature and cost-effectiveness compared to other fillers is evident. Silica G-60 column applications involved the analysis of collected eluents using TLC, and eluents indicating the presence of resveratrol were further analyzed using HPLC-PDA. The HPLC chromatograms of both the eluent (black) and the resveratrol standard (red), as well as the UV spectrum and peak purity analysis, are presented in Fig. 7. The collected eluents from the Silica G-60 column and the presence of resveratrol are presented in the following Table S1.

The HPLC-PDA analysis results of collected fractions showed that eluents 15 and 16 contained *trans*-resveratrol concentrations of 6,6 mg/L and 3,63 mg/L, respectively. The highest concentration was found in eluent 15. Fig. 7 presents the HPLC-PDA chromatogram for eluent 15, along with peak purity. Upon examining the chromatogram, it is evident that many impurities have been removed, particularly noting that most dyes were retained by the column. The critical factor here is the selection of the mobile phase. For *trans*-resveratrol purification in silica columns, the most suitable mobile phase combination was determined to be chloroform, ethyl acetate, and formic acid. In contrast, Sephadex LH-20 was used as another purification method. In a study conducted by Güder et al. (2014) on the isolation of resveratrol from *Vitis labrusca* L., column chromatography using Sephadex LH-20 was discussed. Another study highlighted that Sephadex LH-20, with its cross-linked polysaccharide network, contains numerous active sites for interaction with components in complex mixtures, requiring careful selection of mobile phase compositions for effective chromatographic purification (Piyaratne et al., 2022). Table S2 presents the collected eluents and the presence of resveratrol for Sephadex LH-20 column applications. Examination of HPLC-PDA chromatograms for collected fractions revealed the presence

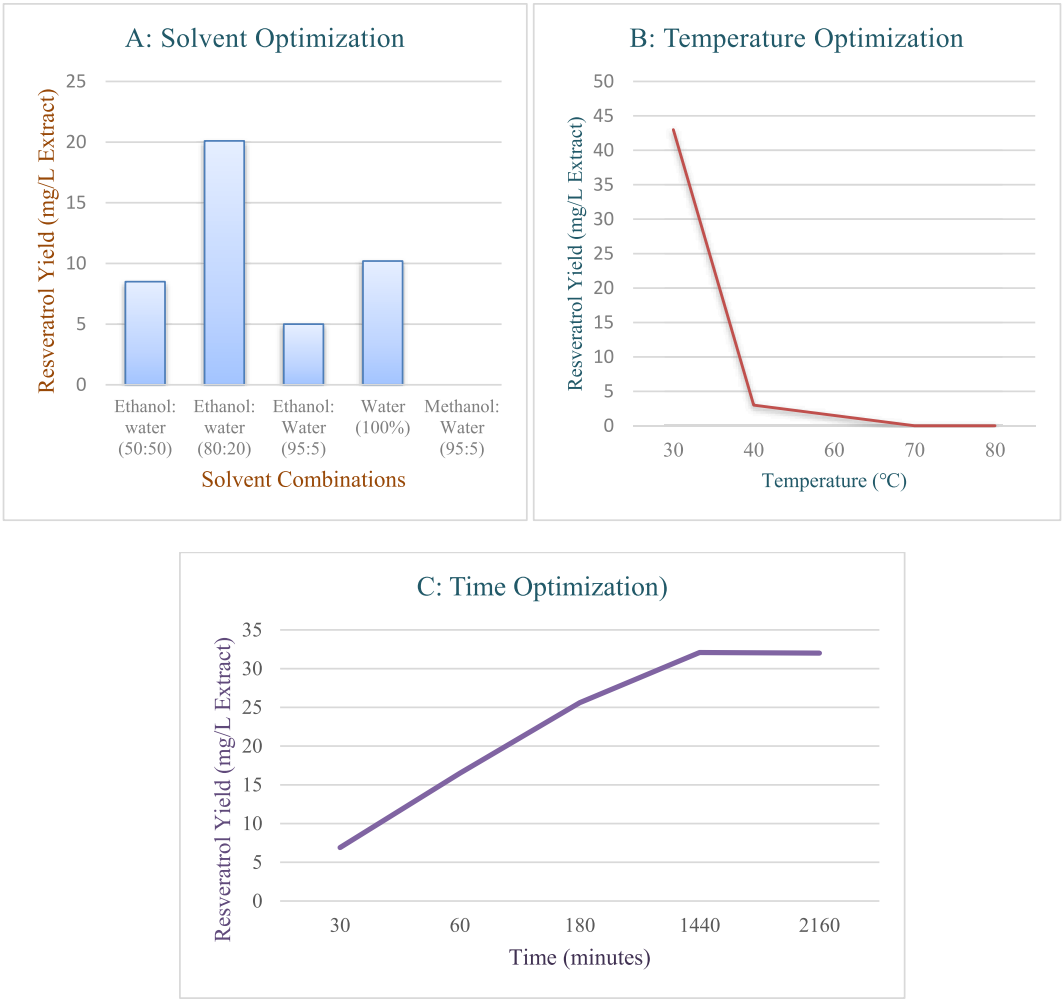

Fig. 5. potential solvent (A), temperature (B), and extraction time (C).

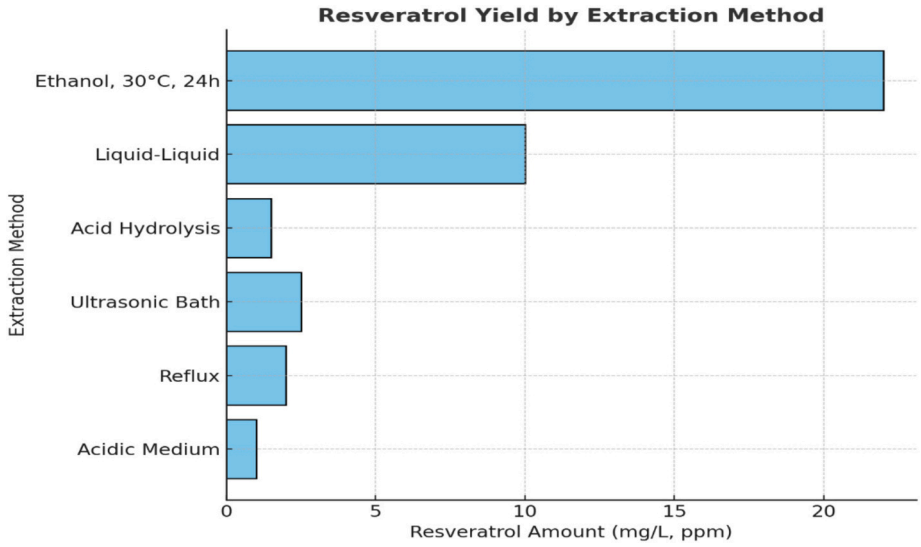

Fig. 6. Resveratrol yield by various extraction techniques.

of *trans*-resveratrol in eluent 4. Fig. 8 provides the chromatogram and peak purity for eluent 4. From the chromatogram, it is evident that Sephadex column application has been highly effective in removing impurities, ensuring the attainment of pure *trans*-resveratrol.

3.5. *Flash chromatography results*

The data obtained through the use of SiliaSep™ HP 12 g with a hexane/ethyl acetate solvent system showed a slight degree of

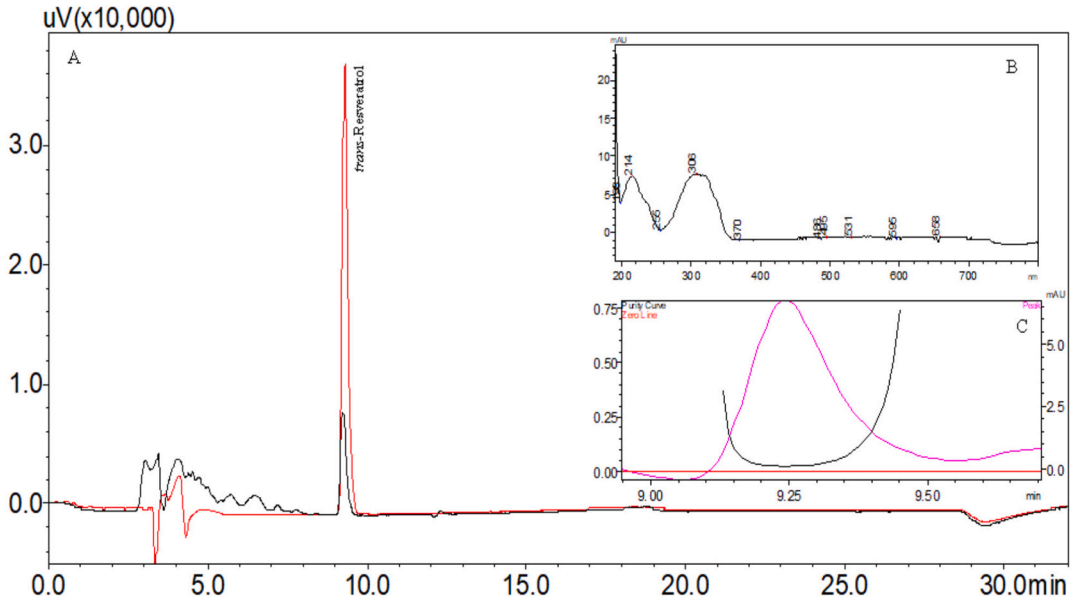

Fig. 7. 15. HPLC chromatogram of eluent (black) and resveratrol standard (red), 15. Eluent(A), UV spectrum (B) peak purity (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

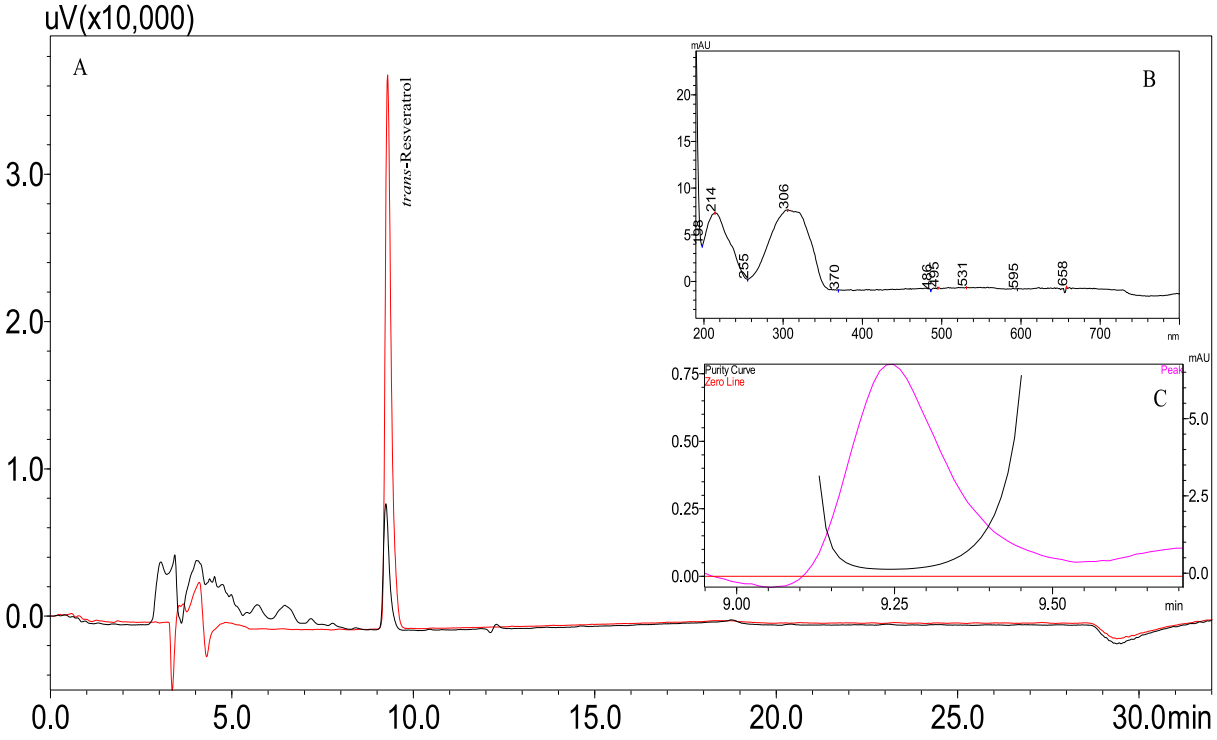

Fig. 8. Chromatogram (A) of eluent number 4 (black) and *trans*-resveratrol standard (red), UV spectrum (B) peak purity of eluent number 4. (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

purification, but it was evident that dye and impurities were not effectively removed (Fig. S2). Fig. S3 presents a trial using SiliaSep™ HP 12 g cartridges. In contrast, the method utilizing SiliaSep™ C_{18} 12 g cartridges with a water/methanol solvent system yielded better results. Initial attempts showed no separation, indicating that resveratrol came with impurities, prompting the decision to proceed with flash chromatography using SiliaSep™ C_{18} 12 g cartridges and a water/methanol solvent system. Fig. S4 shows the flash chromatography data, with five fractions collected. These fractions were analyzed using HPLC-PDA to assess resveratrol content. Table S3 lists the collected eluents and the presence of resveratrol. The results of flash chromatography revealed some effectiveness in removing impurities, though further optimization is needed. The peaks corresponding to the same retention time as the standard were analyzed for UV spectrum and peak purity. As seen in Fig. S5, did not meet the desired threshold, as the purity value was found to be 0.89. Ideally, the peak purity should be within the range of 0.99–1.00, indicating the presence of impurities and interference within the fraction.

3.6. LC-MS/MS mass determination results

The identification and characterization of active compounds in natural substances are crucial for understanding their therapeutic potential. In this study, LC-MS/MS technique was utilized to analyze resveratrol, an effective compound found in grape pomace. The precursor ion of *trans*-resveratrol ($[M–H]^-$, neutral molar mass: 228.24 g/mol) was detected at *m/z* 227.07 and sent to the second quadrupole at a collision energy of 19 V, where it fragmented into characteristic ions with m/z values of 185.25, 143.17, 119.05, and 117.03 through the aid of a collision gas. These fragmentation products are shown in Fig. 9. Fig. S3 presents the LC–MS/MS data, while Table 5 lists the positive and negative ion values related to *trans*-resveratrol.

3.7. H-NMR results

The ^{1}H NMR spectrum of the isolated *trans*-resveratrol compound reveals specific chemical shifts characteristic of its structure. In the spectrum, the methyl peaks from the CD_3OD-d_6 solvent are observed at a chemical shift of 3.35 ppm. Aromatic benzene ring multiplet peaks are noted in the chemical shift range of 6.11–6.98 ppm. The chemical shift for the aliphatic = C–H doublet is observed between 7.36 and 7.40 ppm, and the aromatic benzene ring's phenolic O—H proton peak is seen at 9.24 ppm. The integration ratios of these peaks confirm the results. Fig. 10 displays the ^{1}H NMR chemical shift (δ) values (ppm) for isolated *trans*-resveratrol.

3.8. Preparation and characterization of liposomal formulations

The lyophilization technique has been reported as the most commonly used method in the production of pharmaceutical products that are heat-sensitive and unstable in aqueous environments, in order to ensure stability in dried form and long-term storage. (Degobert & Aydin, 2021). Formulations prepared with lecithin were synthesized as nanoformulations with particle sizes smaller than 157 nm (Table 6). Particle size is a critical factor in the development of liposomes, as it directly influences the stability of colloidal systems, their biological distribution, and drug release properties (Mozafari et al., 2008).

The polydispersity index (PDI) provides quantitative information about the homogeneity and size distribution of vesicles in liposomal suspensions (Vanaja et al., 2013). PDI values of 0.5 and above indicate a heterogeneous particle size distribution within the system. In this context, the formulation obtained in this study was evaluated to have a PDI below 0.5 (Table 6). Additionally, the incorporation of RES with lecithin resulted in a lower PDI value compared to the control group. Zeta potential analysis demonstrated that the control formulation (L-1, lecithin only) exhibited a high negative surface charge with an average value of-73.4 mV, indicating excellent colloidal stability consistent with previous studies reporting zeta potentials below −30 mV as indicative of stable nanoparticle dispersions (Honary & Zahir, 2013). Upon encapsulation of resveratrol (L-2), the zeta potential decreased to-56.6 mV, reflecting partial surface charge neutralization likely due to the incorporation of the relatively hydrophobic resveratrol molecules on or within the lecithin vesicles. Despite this decrease, the zeta potential remained sufficiently negative to maintain electrostatic stability, confirming the colloidal integrity of the encapsulated system (Joye et al., 2015). Dynamic Light Scattering (DLS) analysis results revealed significant differences in particle size and distribution between the control formulation (L-1, lecithin only) and the resveratrol-loaded formulation (L-2) The *Z*-Average (mean hydrodynamic diameter) increased from 359.7 nm in L-1 to 442.1 nm in L-2. This notable increase indicates that resveratrol was successfully incorporated into the lecithin-based system, resulting in a significant enlargement of the vesicle size. The incorporation of polyphenolic compounds into lipid bilayers leading to an increase in particle size has also been reported in the literature. In particular, reported a similar increase in particle size when polyphenols derived from grape seeds were loaded into solid lipid nanoparticles (Trapani et al., 2022).

Table 5
Positive and negative ion fragments of the *trans*-resveratrol molecule.

Positive ion values	Negative ion values
196,541	195,541
215	226,4
228	278,229
255	

3.9. Encapsulation efficiency

The encapsulation efficiency of resveratrol in lecithin nanoparticles with a 1/1 and 1/2 core:coating ratio was found to be 65.0 % (±1.0 %) and 80.0 % (±1.05 %), respectively. Various encapsulation methods have been used for resveratrol, showing varying efficiencies. For instance, nanoparticles coated with poly(D, L-lactide-*co*-glycolide) exhibit encapsulation efficiencies ranging from 17 % to 33 % (Sanna et al., 2012), and calcium-alginate microparticles encapsulate resveratrol with efficiencies reaching up to 98 % (Cho et al., 2014). Katja et al. (2015) encapsulated resveratrol into Ca-alginate submicron particles, achieving an efficiency of 24.5 % (±1.6 %). They reported that the encapsulation efficiency is closely related to the preparation method. In a study by Huang et al. (2019) and Wang et al. (2022), lecithin-based encapsulation of resveratrol increased its solubility, achieving an encapsulation efficiency of 65.45 %. In our study, lecithin encapsulated resveratrol, despite not dissolving in water, was able to form micelles in

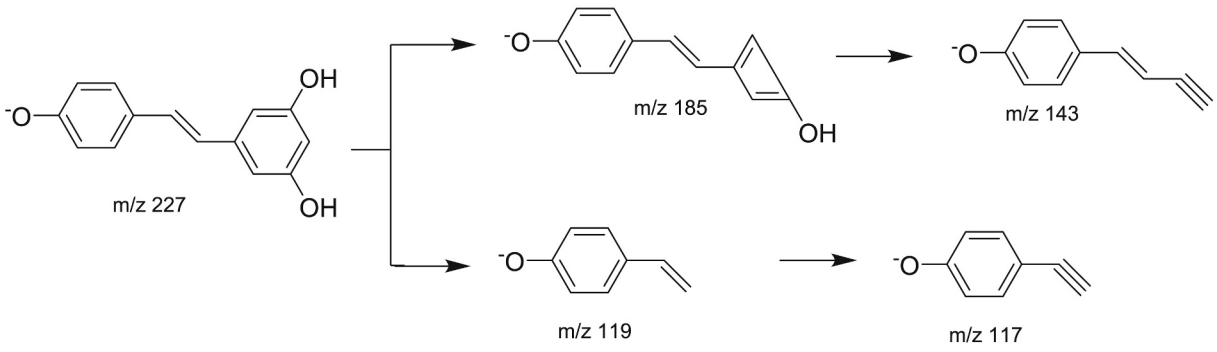

Fig. 9. *Trans*-resveratrol fragmentation products.

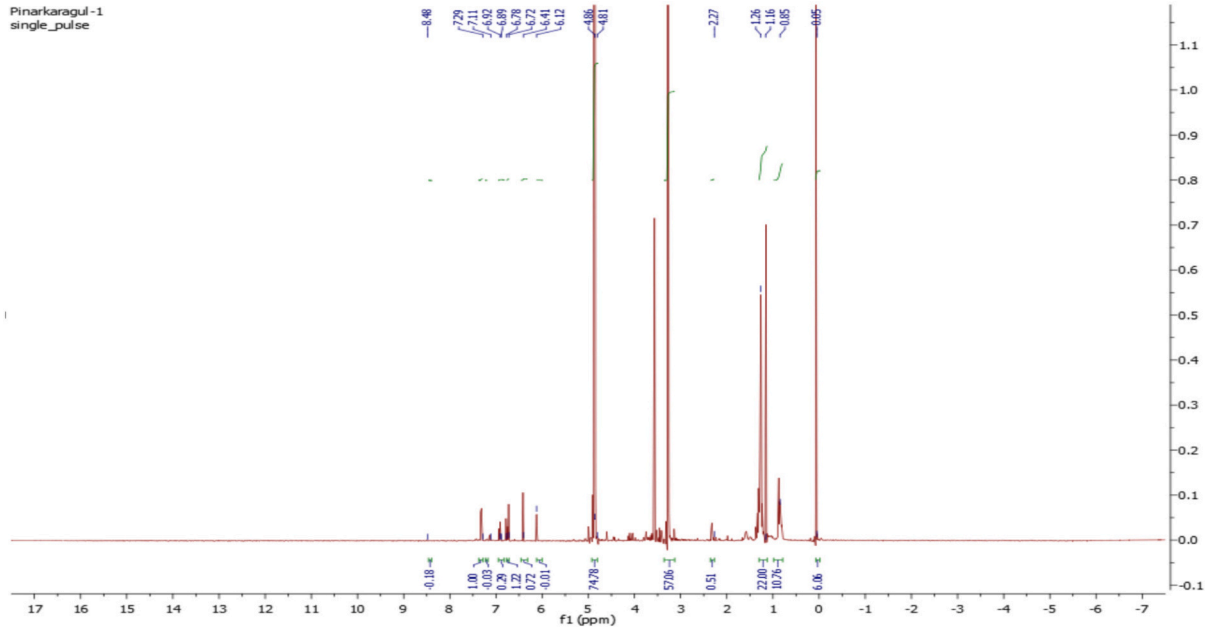

Fig. 10. ^{1}H NMR chemical shift (δ) values of isolated *trans*-resveratrol (ppm). ^{1}H NMR (500 MHz,) δ 7.37–7.27 (m, 1H), 7.17–7.04 (m, 1H), 6.96–6.88 (m, 1H), 6.78–6.68 (m, 1H), 6.45–6.34 (m, 1H), 6.13–6.04 (m, 1H), 5.04–4.92 (m, 1H).

Table 6
Zeta potential, particle size, and polydispersity index (PDI) values of nanoparticle formulations: L-1 corresponds to the control group (lecithin only), and L-2 represents the resveratrol-loaded nanoparticles prepared with lecithin.

Sample	Zeta Potential (mV)	Particle size (nm)	Polydispersity Index (PDI)	Stability Interpretation	*Z*-Average (nm)
L-1	−73.4 ± 6.0	263,1	0,214	Very stable, moderate size uniformity	359,7
L-2	−56.6 ± 6.1	157,5	0,075	Stable, highly uniform	442,1

aqueous environments, thereby significantly enhancing its bioavailability.

3.10. Morphological analysis

The surface structures of encapsulated resveratrol microparticles analyzed using Scanning Electron Microscopy (SEM) images are crucial for evaluating their physical properties. These SEM images have been provided in the Supplementary Material. The surface morphology revealed that the microparticles have a rough surface, suggesting an increased surface area, which could positively influence controlled release behavior. Additionally, the absence of any crystalline structure on the microparticle surfaces indicates that a significant portion of resveratrol is retained within the capsules. In a study by Lei et al. (2022), lecithin-encapsulated resveratrol was observed in microparticle form, showing needle-like structures. In our study, instead of needle-like structures, spherical shapes were observed, indicating successful preparation of lecithin-coated resveratrol microparticles. SEM analysis of pure lecithin revealed repulsion between hydrophilic groups, whereas in trans-resveratrol-loaded lecithin capsules, polar regions appeared to interact, promoting structural integrity The obtained SEM images are provided in the Supplementary Information: Fig. S4 (30 μm), Fig. S5 (100 μm), and Fig. S6 (300 μm).

3.11. In vitro release studies

Resveratrol's release profiles in simulated gastric fluid (HCl/KCl, pH 1.5) and simulated intestinal fluid (NaH_2PO_4, pH 6.8) aqueous solutions are shown in Figs. 12 and 13, respectively. The release profile in the HCl/KCl environment (pH 1.5) indicates that the release of free *trans*-resveratrol in the digestive system is significantly low. This is attributed to the low solubility of resveratrol in the acidic stomach environment. The release profile in the NaH_2PO_4 environment (pH 6.8) shows that encapsulated resveratrol completely dispersed in artificial small intestine fluid after 2 h, releasing all active components. The release in the stomach environment showed better results compared to free resveratrol, but still did not reach the desired level. These results suggest that encapsulated resveratrol exhibits better solubility in the intestinal environment, while remaining stable in the stomach environment. Upon reaching the intestinal environment, complete release occurs, allowing for absorption from the gut. Figs. 11 and 12 illustrate the release profiles of encapsulated resveratrol and free resveratrol, respectively.

As shown in Figure 11, the release of resveratrol molecules encapsulated with lecithin exhibited a steady increase, reaching approximately 5 % within the first 5 min. After this point, the release showed a slight increase, reaching around 25 %, and then stabilized. In contrast, non-encapsulated resveratrol remained insoluble in the medium. These findings indicate that the solubility of encapsulated resveratrol is superior to that of non-encapsulated resveratrol; however, it still does not reach the desired level.

As shown in Fig. 12, the release of resveratrol molecules encapsulated with lecithin exhibited a steady increase of approximately 20 % within the first 90 min, reaching nearly 80 % by the 120th min. In contrast, non-encapsulated resveratrol remained insoluble in the medium. These findings suggest that the solubility of encapsulated resveratrol is enhanced in the intestinal environment. Resveratrol is a significant phytoalexin with a long history of use, and numerous studies have highlighted its health benefits. Despite its therapeutic effects, the clinical application of resveratrol is limited due to its low bioavailability and rapid metabolism. To address these challenges, nanoemulsion-based delivery systems offer a promising approach by improving the solubility of the bioactive compound, enhancing absorption, and enabling

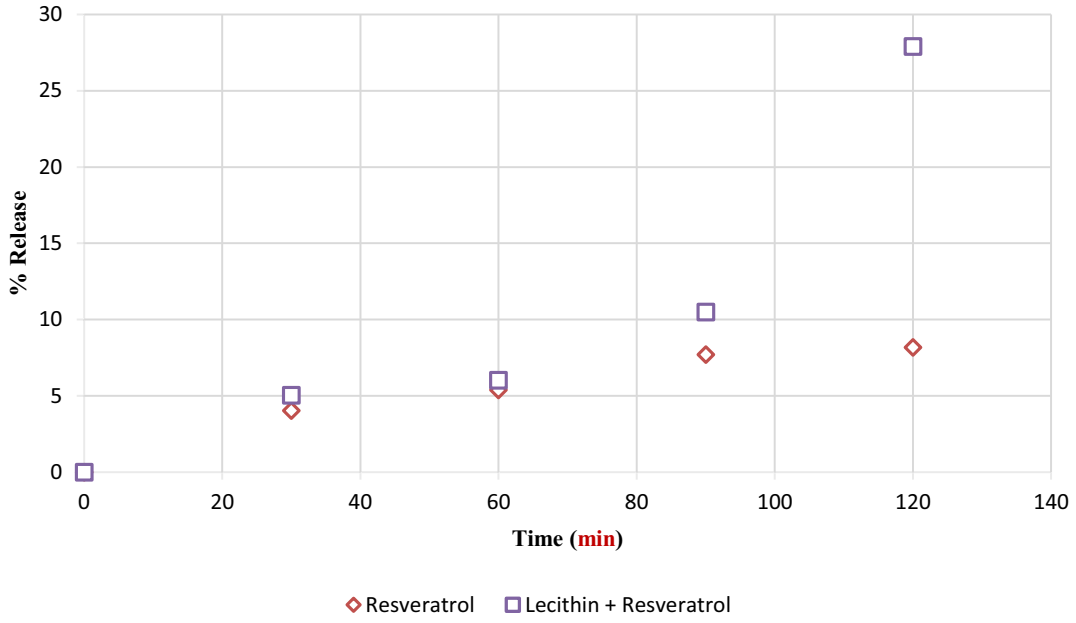

Fig. 11. Release of *trans*-resveratrol and Lecithin + *trans*-resveratrol in Stomach Environment (%).

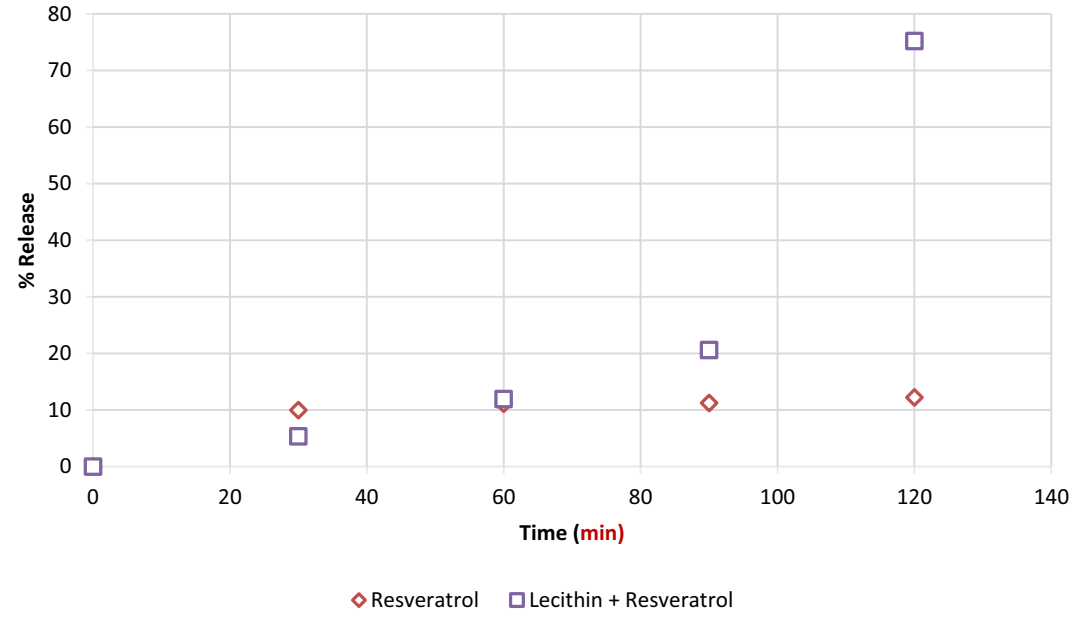

Fig. 12. Release of Resveratrol and Lecithin + Resveratrol in Intestinal Environment (%).

controlled release to target regions effectively. Such systems hold potential for optimizing the therapeutic efficacy of resveratrol. In a study conducted by Sessa et al. (2011), submicron nanoemulsions produced via high-pressure homogenization (HPH) demonstrated the ability to protect resveratrol from chemical degradation and prevent its oxidation to inactive forms, such as *cis*-resveratrol. Additionally, optimized nanoemulsion formulations enabled resveratrol to remain encapsulated within the lipid phase during digestion, reach the colon without being metabolized in the gastrointestinal tract, and be efficiently absorbed through the intestinal wall. These O/W nanoemulsions, containing dual emulsifiers, were concluded to be effective carrier systems for improving the bioavailability of bioactive compounds due to their enhanced protective properties (Sessa et al., 2011). The low bioavailability and rapid excretion of resveratrol limit its clinical application (Francioso et al., 2014). Therefore, an effective carrier system is required to improve its efficacy. Liposomes, lipid-based drug delivery vehicles, have been extensively studied and widely used in various biomedical applications (Bhattacharyya et al., 2014). During the selection of encapsulation methods, techniques such as spray drying were tested. However, due to the heat sensitivity of resveratrol, lyophilization was chosen. The optimal lyophilization duration was determined to be 3 days. The use of coating materials significantly improved the storage stability of resveratrol. In an in vitro simulated gastrointestinal digestion model, it was found that continuous release of resveratrol from lecithin-encapsulated resveratrol (Lecithin-RES) capsules occurred more easily. Moreover, its solubility in water increased significantly, particularly in the intestinal environment, where high levels of resveratrol dissolved within the first 30 min, achieving near-complete dissolution and release by the end of one hour.

4. Conclusions

Vitis vinifera byproducts are a significant source of bioactive polyphenolic compounds beneficial to human health, and the valorization of such waste materials is gaining increasing importance in terms of sustainability. In this study, *trans*-resveratrol was extracted and purified under optimal conditions from grape pomace and encapsulated with lecithin; its stability and release behavior were evaluated in an in vitro simulated gastrointestinal environment.

The chemical structure of resveratrol was confirmed using various analytical techniques (LC-MS/MS, HPLC-PDA, and NMR), and purification was achieved through innovative methods such as flash chromatography. As a result of encapsulation, a system that enhances bioavailability was developed, supported by particle size increase, morphological structure, and zeta potential analysis. In vitro release studies demonstrated that lecithin encapsulation promoted the controlled and pH-dependent release of *trans*-resveratrol (RES). These results suggest that grape pomace waste can be transformed into valuable natural resources for use in the food and pharmaceutical industries.

However, this study has certain limitations. Firstly, in vitro release tests may not fully reflect the complexity of the real gastrointestinal system. Furthermore, no in vivo studies have been conducted to assess bioavailability and metabolic transformation. The long-term stability of the encapsulation under storage conditions has also not been comprehensively evaluated. Future studies are recommended to address these limitations by including in vivo pharmacokinetic analyses, long-term stability testing, and comparisons of different carrier systems.

In this context, although this study represents an important step toward enhancing the functional use and bioavailability of *trans*-resveratrol obtained from grape pomace, it should be supported by more comprehensive research.

CRediT authorship contribution statement

Pınar Karagül: Writing – original draft, Software, Methodology, Data curation. **Halil İbrahim Uğraş:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.146352.

Data availability

Data will be made available on request.

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