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루테인/지아잔틴이 풍부한 계란 노른자 분말의 분무 건조 및 동결 건조 방법 비교: 마요네즈 제형에서의 적용

Comparison of spray-dryingand freeze-drying methods for lutein/zeaxanthin-enriched egg yolk powder andtheir application in mayonnaise formulation

중화음, 우정초, 오연진주, 신원선*
한양대학교 식품영양학과
Hexin Zeng, Jingchao Yu, Yingjinzhu Wu, Weon-Sun Shin*
Department of Food and Nutrition, College of Human Ecology, Hanyang University

Abstract

The absence of carotenoids in the diet leads to ocular issues, such as cataracts, which may be mitigated by supplementing foods with lutein and zeaxanthin (L/Z), the macular pigments. This study investigated the impact of spray-drying (SD) and freeze-drying (FD) on the physicochemical properties of L/Z-enriched egg yolk (SDE, FDE). The results revealed that FDE contained a significantly higher concentration of L/Z (33.05±1.62 μg/g) compared to SDE (25.59±0.09 μg/g). Although SDE exhibitedsuperior solubility than that in FDE, the latter demonstrated higher oil holding capacity (OHC, 3.34 □ 0.23 g/mL) and emulsifying stability index (ESI, 493.92±68.46 min) across all samples. Additionally, sensory evaluation indicated that mayonnaise prepared with L/Z-enriched yolk powder (SDE, FDE) was more acceptable to panelists than those made with control yolk powder (SD, FD), suggesting that L/Z-enriched yolk powders could be effectively used as emulsifying agents in food products.

Key words : *lutein, zeaxanthin, freeze-drying, spray-drying, egg yolk powder, emulsion*
주제어 : 루틴, 지아잔탄, 동결건조, 분모건조, 계란 노른자 분말, 에멀젼

Ⅰ. Introduction

Lutein and zeaxanthin (L/Z) are carotenoids commonly referred to as macular pigments (MPs). These pigments selectively accumulated in the macula of the human retina (Landrum & Bone, 2001), where they provide protective effects against light-induced retina damage (Roberts & Dennison, 2015). MPs function as natural filters of blue light, reducing phototoxicity caused by high-energy blue light (Junghans et al., 2001). Zeaxanthin is concentrated in the central macula, while lutein is distributed more broadly across the retina (Bernstein et al., 2016). However, as L/Z cannot be synthesized endogenously in humans, they must be obtained through dietary intake (Kim & Shin, 2022; Xu et al., 2022).

Green leafy vegetables, including kale, spinach, broccoli, peas and lettuce as well as egg yolks, are significant dietary sources of L/Z (Abdel-Aal et al., 2017). Although egg yolks contain relatively lower concentrations of MPs compared to some plant sources, they are considered superior due to their enhanced bioavailability of L/Z. The high-fat content and lipid matrix of egg yolks facilitate the absorption of carotenoids, making them an optimal medium for delivering L/Z in the human diet (Tsiaka et al., 2018). Regular consumption of eggs has been shown to significantly elevate serum L/Z labels, suggesting that eggs could play a role in preventing cataracts and age-related macular degeneration (AMD)—the leading cause of irreversible blindness in adults (Kishimoto et al., 2017, Ma et al., 2016).

MP-enriched eggs can be produced by supplementing the diet of the laying birds with natural carotenoids sources, such as alfalfa concentrate, tomato powder and marigold extracts (Karadas et al., 2006) (Jeon et al., 2012). Among these, microalgae offer distinct advantages, including rapid growth

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* Corresponding Author ; Weon-Sun Shin
Tel : 82-2-2220-4204, E-mail : hime@hanyang.ac.kr

rates, year-round availability, and reduced labor intensity, making them a cost-effective source of lutein (Fernández-Sevilla et al., 2010). For instance, supplementing hen diets with a small amount (0.3%) of dried *Chlamydomonas reinhardtii* mutant has been reported to double the MP concentration in egg yolks (Baek et al., 2018). Additionally, *Dunaliella* species characterized by the absence of indigestible cell walls, are promising feed sources for carotenoid enrichment (Ben-Amotz, 1980). Mutants of *Dunaliella tertiolecta*generated by ethyl methanesulfonate (EMS) treatment have demonstrated enhanced zeaxanthin content, offering potential for both egg enrichment and commercial zeaxanthin production (Kim et al., 2017).

Egg yolks are widely used in food industries for their functional properties, including foaming, coagulation, emulsification, and coloring. However, the fresh egg yolks are perishable and challenging to transport. To address these issues, the food industry increasingly favors dried egg products over liquid eggs due to their extended shelf life, reduced microbial risks, and elimination of refrigeration requirements (Miranda et al., 2015, Jesús et al., 2013). Egg yolk powder is particularly valued in bakery products, mayonnaise, salad dressings, ice cream, pasta, and various convenient foods due to its stability, emulsifying capacity, and ability to enhance product texture (Asghar & Abbas, 2012). Mayonnaise, a semi-solid oil-in-water emulsion typically prepared with eggs, vinegar, and mustard, is among the most widely consumed products incorporating egg yolks (Kishk & Elsheshetawy, 2013). While efforts to develop healthier mayonnaise formulations have often focused on reducing fat content, limited research has explored the enrichment of L/Z in egg yolks to enhance the nutritional value of mayonnaise and potentially mitigate AMD risk (Motta-Romero et al., 2017).

Among various food preservation techniques, drying is one of the most widely accepted methods due to its ability to extend shelf life, reduce weight, and enhance convenience (Chhabra et al., 2024). Spray-drying and freeze-drying are two commonly employed drying methods, each with distinct mechanisms and outcomes. Spray-drying involves atomizing a liquid feed into a hot drying medium, where rapid moisture evaporation produces a free-flowing powder (Marante et al., 2020). However, this method often causes degradation of volatile and heat-sensitive compounds (Samborska et al., 2019). To address these limitations, freeze-drying has emerged as an advanced alternative. This method freezes the material and removes water through sublimation (primary drying) and desorption (secondary drying), preserving heat-sensitive compounds more effectively while maintaining the structural integrity of the product (Meera et al., 2016).

Despite significant advancements in understanding the effects of pasteurization, drying, and storage on xanthophyll content in egg yolks (Wenzel et al., 2010, Franke & Kießling, 2002), limited research has focused on the comparative impact of different drying methods on lutein and zeaxanthin (L/Z) retention. Additionally, the influence of these drying methods on the physicochemical properties of egg yolk powder, such as solubility, water/oil holding capacity, and emulsion stability upon reconstitution, remains underexplored.

Given the rising interest in MP-enriched foods, this study aimed to evaluate the effects of spray-drying and freeze-drying on the L/Z content and physicochemical properties of MP-enriched egg yolk powder. Furthermore, our study investigated the application of these powders in mayonnaise, to assess how drying methods influence functional performance and sensory attributes. Our research seeks to optimize both nutritional and functional properties of MP-enriched yolk powder for broader food industry applications.

Ⅱ. Materials and Methods

2.1 Materials and reagents

Laying hens (Hy-line Brown, aged 29 weeks) were sourced from a local farm in Seoul, Korea. The hens were randomly divided into two groups: a control group fed a standard commercial diet and an experimental group fed a diet supplemented with 0.2% Dunaliella tertiolecta mp3 powder. The feeding experiment was conducted over a period of three weeks, and eggs were collected daily from both groups. All eggs used for the four experimental sample groups were obtained within the same period to ensure consistency across treatments.

The *Dunaliella tertiolecta mp3* powder used in this study was provided by Prof. Eon-Seon Jin of Hanyang University. This microalga is an ethyl methanesulfonate (EMS)-induced mutant strain with enhanced zeaxanthin content, as reported by Kim et al. (2017). The strain was cultivated, harvested, and freeze-dried into powder form before use. The supplementation level of 0.2% was determined based on prior studies (Baek et al., 2018), which demonstrated its efficacy in enriching egg yolks with lutein and zeaxanthin while maintaining the health and productivity of the hens. Safety assessments for the use of this microalga as a feed supplement have been reported in previous research. The safety of this algae for animal consumption has been previously evaluated in accordance with established guidelines, and the resulting eggs were processed under pasteurization conditions (60 ℃, 3.5-5 min) before any sensory evaluation to ensure food safety.

Because the study involved only dietary changes under standard farming conditions and did not involve animal testing procedures or drug administration, it did not require ethical approval from an Institutional Animal Care and Use Committee (IACUC). In addition, the use of these egg yolk samples in sensory evaluation was approved by the Institutional Review Board (IRB) of Hanyang University, confirming their safety for human consumption.

2.2 Egg yolk drying

2.2.1 Freeze-drying

Freeze-drying was performed using a laboratory freeze dryer (ILSHIN BIOBASE, freeze dryer-FD 850). Eggs from both the control and L/Z-enriched groups were washed with water, cracked open, and the yolks were manually separated from the egg whites. The egg yolks were homogenized using an Ultra-Turrax T25 (IKA, Staufen, Germany) at 3,000 rpm for 30 s and transferred into sterilized bottles. The homogenized samples were pasteurized in a water bath at 60 ℃ for 3.5 min, rapidly cooled in an ice-water bath, and subsequently transferred into sterilized containers. The samples were then frozen at -80℃ for 24 h and freeze-dried under conditions of approximatively -80℃ and 5.33 Pa. The freeze-dried control group (FD) and enriched group (FDE) were ground to a fine powder using a mortar, packed in sealed plastic bags, and stored under refrigerated until further analysis.

2.2.2 Spray-drying

For spray-drying, the control and enriched egg yolks were processed to prepare spray-dried enriched egg samples (SDE). In the SDE preparation, gelatin (2.27% w/w), lactose, distarch phosphate, Arabic gum, dextrin (12 DE) at concentrations of 1.81% (w/w) were added as carrier substances. In contrast, no carrier substances were added to the control sample (SD). Each sample was homogenized at 3,000 rpm and 60 ℃ for 5 min (Ultra-Turrax T25, IKA, Staufen, Germany) and subsequently sterilized in a water bath for 15 min. Prior to drying, the sample temperatures were kept at 40 ℃. The powder was obtained using a spray dryer (Ohkawara L-8; Ohkawara Kakohki Co., Ltd., Yokohama, Japan) with inlet and outlet air temperatures set to 155-170 ℃ and 90-105 ℃, respectively. The resulting powders were packed into commercial bags (approximately 200 g) and stored under refrigerated until further utilization.

Four yolk powders were prepared using different drying methods and enrichment conditions. SD (spray-dried yolk powder (non-enriched)), FD (Freeze-dried yolk powder (non-enriched)), SDE (Spray-dried lutein/zeaxanthin-enriched yolk powder), FDE (Freeze-dried lutein/zeaxanthin-enriched yolk powder).

2.3 Extraction of lutein/zeaxanthin

To extract the pigments, fresh egg yolks (0.5 g) were mixed in 10 mL acetone (90% v/v) and mixed adequately with continuous stirring. The solution was then centrifuged at 2,500 × *g* for 2 min, and the supernatants were filtered with 0.2 μm nylon filters (Sigma Aldrich Inc, St. Louis, Missouri, USA) and subjected to high-performance liquid chromatography (HPLC) analysis. Aliquots (0.5 g) of egg yolk powder were placed directly into falcon centrifuge tubes (50 mL), then 13 mL of methanol was added and covered to avoid solvent evaporation. The solution was immediately treated with ultrasound for 60 s to improve extraction. Subsequently, the samples were homogenized using an Ultra-Turrax T25 (IKA, Staufen, Germany) at 4,000 rpm for 2 min. After an incubation of 20 min, the samples were centrifuged (Combi R-514, Hanil Scientific Inc., Incheon, Korea) at 2,500 × *g* at 20 ℃ for 5 min. Then the aliquots of the supernatants were directly placed in HPLC vials using a plastic syringe (1 mL) and a nylon filter (0.2 μm) (Wenzel et al., 2010). The mobile phase for HPLC analysis system consisted of 0.1 M Tris-HCl (pH

8.0)-acetonitrile-methanol (14:84:2, v/v/v) for the first 15 min and acetonitrile-methanol (32:64, v/v) for the last 5 min. The injection volume was 20 μL and the flow rate was 1.2 mL/min. The column oven temperature was set to 40 ℃, and L/Z were detected at 445 nm.

2.4 Solubility

Twenty-five milliliters of distilled water was added to the egg yolk powder sample (0.25 g) and transferred into a centrifuge tube. The solution was homogenized at 5,000 rpm for 5 min followed by centrifugation at 3,000 × *g* for 5 min. An aliquot of 5 mL of the supernatant was then transferred to a pre-weighed beaker and immediately oven-dried at 105 ℃ for 5 h. The solubility (%) was calculated as the weight difference using Eq. (1).

$$Solubility(\%) = \frac{W1}{W2} \times 100 \qquad (1)$$

W1: Weight of powder in the supernatant (g)

W2: Weight of podwer in the solution (g)

2.5 Determination of water holding capacity and oil holding capacity (WHC/OHC)

The WHC/OHC was measured using a method described in a previous study with a slight modification (Zhang & Li, 2009). Briefly, 0.5 g (M_1) sample was transferred to a pre-weighed centrifuge tube (M), and 7 mL of distilled water was added. The sample containing centrifuge tubes were then placed in a water bath at 60 ℃ and held for 10 min, followed by centrifugation at 2,500 × *g* for 15 min. The supernatant was removed, and the centrifuge tubes with the wet powders were weighed (M_2). WHC was calculated using Eq. (2).

$$WHC(g/g) = \frac{M2 - (M1 + M)}{M1} \qquad (2)$$

To measure OHC, 1 g sample (M) was mixed with 10 mL rapeseed oil (V_1) in a 50 mL centrifuge tube. The solution was stirred with a vortex mixer (Vortex-Genie 2) for 30 s every 5 min and continued for 30 min. Subsequently, the solution was centrifuged at 3,000 × *g* for 25 min. The free oil (V_2) in the centrifuge tube was removed, and the absorbed oil was determined by the difference between V_1 and V_2. The oil holding capacity was calculated using Eq. (3).

$$OHC(mL/g) = \frac{V1 - V2}{M} \qquad (3)$$

2.6 Preparation of mayonnaise

Mayonnaise was prepared based on the recipe described by Jeong et al. (2021) with a slight modification. The formulation consisted of the following ingredients (in percentages): corn oil (70%, w/v), egg yolk powder (SD, FD, SDE, FDE) (5%, w/w), water (15%, w/v), sugar (5%, w/w), vinegar (3.5 %, w/v), and salt (1.5%, w/w). SD and FD are egg yolk powders from non-enriched yolks (spray-dried and freeze-dried, respectively) and were used to prepare control mayonnaise. SDE and FDE are from Dunaliella tertiolecta-enriched yolks and were used to prepare L/Z-enriched mayonnaise. All ingredients, except for corn oil, were mixed and homogenized at 3,000 rpm for 60 s. Subsequently, corn oil was gradually incorporated into the mixture while homogenizing was continued at 4,000 rpm for 5 min. After the complete addition of the oil, the emulsion was further homogenized at 5,000 rpm for additional 2 min to ensure uniformity.

2.7 Emulsifying activity index (EAI) and emulsion stability index (ESI)

EAI and ESI were determined according to the method of Hernández-García et al. (2016) and Jeong et al. (2021)with a slight modification. Briefly, the emulsion was prepared by mixing 1 g yolk powder with 10 mL distilled water and 10 mL corn oil in a calibrated centrifugal tube. The emulsion was homogenized at 13,600 rpm for 2 min using a homogenizer (Ultra-Turrax T25 IKA, Staufen, Germany). An aliquot of the emulsion (20 ìL) was pipetted from the bottom of the tube at 0 and 10 min after homogenization and diluted to 2 mL with 0.1% SDS solution (1:100 dilution). The absorbance of the diluted solution was read at 500 nm using a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis Spectrophotometer, Berryville, Virginia), and 0.1% SDS solution was used as the blank. The EAI and ESI were calculated by Eq. (4) and Eq. (5), respectively.

$$EAI(m2/g) = \frac{2 * 2.303 * dil * A0}{c * 10000 * \theta} \qquad (4)$$

Where ‘dil’ is the dilution factor (100); ‘A’ is the absorbance at 500 nm; ‘c’ is the egg powder concentration (g/mL), 0.01; and è is the dispersed phase volume fraction (0.5).

$$ESI(\min) = A0 * \frac{\Delta t}{\Delta A} \qquad (5)$$

where ÄA = A_0 − A_{10} and Ät = 10 min, A_{10} and A_0 represent the absorbance after 10 min and time zero, respectively, at 500 nm.

2.8 Color measurement

Color values (L^*, a^*, b^*) of freeze-dried yolk powder, spray-dried yolk powder and mayonnaise prepared with powdered yolk were measured in triplicate by a colorimeter (Konica Minolta Chroma Meter CR-400, Minolta Italia S.p.A., Milano, Italy) using illuminant source C. The equipment was calibrated using a white standard ceramic tile (Reference No. 1353123. Y=92.7, x=0.3133, and y=0.3193).

2.9 Sensory analysis

The sensory acceptability of the formulated mayonnaise was evaluated by a panel of 20 participants (10 males and 10 females) aged from 20 to 50 years. To ensure the reliability of the data, the panelists received a brief orientation and training session prior to the sensory evaluation. The sensory attributes assessed included color, aroma, taste, stickiness, flavor and overall acceptability. Mayonnaise samples were served in disposable cups, each containing a standardized amount of 5 g. The samples were coded with three-digit random numbers to ensure anonymity, and mineral water was offered for cleaning the palate between the evaluations. Sensory evaluation was conducted using a 9-point hedonic scale, where 1 represented dislike extremely, and 9 represented like extremely.

2.10 Statistical analysis

Each experiment was performed in triplicate, and data are presented as mean ±standard deviation (SD). The mean values from the three separate experiments or replicate analysis were reported. Statistical comparisons of the means were performed using Duncan’s post-hoc test ($p < 0.05$) with SPSS software 23.0 (IBM SPSS Statistics, USA).

III. Results and discussion

3.1 Quantification of L/Z in fresh and processed egg yolk samples

The concentrations of L/Z in yolk powder from the control and L/Z-enriched groups are shown in <Table 1>. To better assess the enhancement effect of *Dunaliella tertiolecta mp3* powder supplementation, reference values from non-enriched fresh egg yolks were considered. According to Chung et al. (2004), control fresh yolk typically contains approximately 0.7 μg/g of lutein and 0.4 μg/g of zeaxanthin, which are significantly lower than those found in the enriched fresh yolk used in this study (12.53 ± 0.40 μg/g for lutein and 12.87 ±0.67 μg/g for zeaxanthin). This clearly demonstrates that dietary supplementation with 0.2% *Dunaliella tertiolecta mp3* powder substantially enhances yolk carotenoid levels, consistent with previous studies (Baek et al., 2018; Jeon et al., 2012).

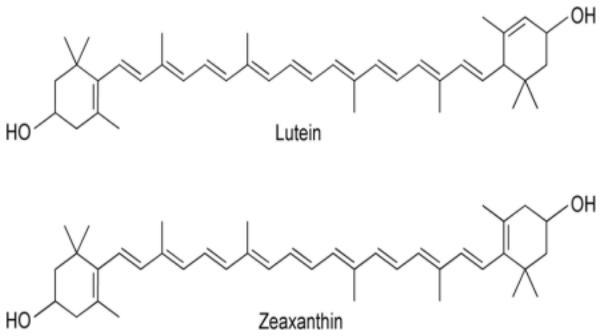
	Enriched fresh yolk	Control dried powder		Enriched dried powder	
		SD	FD	SDE	FDE
Lutein (μg/g)	12.53 ±0.40^c	18.12 ±0.33^b	19.51 ±0.45^a	25.59 ±0.09^b	33.05 ±1.62^a
Zeaxanthin (μg/g)	12.87 ±0.67^c	15.39 ±0.88^b	18.51 ±1.03^a	23.97 ±0.09^b	31.25 ±1.60^a

<Table 1> Lutein and zeaxanthin concentrations in fresh enriched egg yolk, control dried samples and enriched dried samples

The loss of L/Z during freeze-drying (FDE) was lower compared to spray-drying (SDE) in both the control and L/Z-enriched groups. The recovery rate, defined as the yield of egg yolk powder from the weight of fresh liquid yolk (%), was higher in FDE (46.8%)than in SDE (43.5%). Theoretical L/Z contents per gram of dried powder were estimated based on the L/Z contents in fresh enriched egg yolk and the recovery rates of each drying method. When compared to these theoretical values, the actual L/Z contents in SDE were reduced by 11.16% and 18.98%, respectively. Conversely, the actual L/Z contents in FDE exceeded the theoretical values by 23.39% and 13.59%, respectively.

Lutein and zeaxanthin naturally occur as all-trans isomers <Figure 1>; however, under thermal processing and storage conditions, trans-isomers are prone to isomerization into cis-forms, resulting in reduced bioactivity (Sui et al., 2014). The observed losses in SDE can be attributed to thermal degradation and volatilization during high-temperature spray-drying. These changes not only lower L/Z content but also reduce pigment intensity, as reflected in the color analysis.

Interestingly, the L/Z contents in FDE exceeded theoretical estimates. This may be due to improved retention and stabilization of carotenoids during freeze-drying, which minimizes degradation by avoiding high temperatures. In addition, the structural disintegration of yolk components during freezing may enhance pigment extractability during analysis, leading to higher measured concentrations (Franke & Kießling, 2002; Wenzel et al., 2010). The porous structure of freeze-dried powders may also promote the preservation and recovery of hydrophobic compounds like lutein and zeaxanthin (Sogi et al., 2015). Taken together, these findings suggest that freeze-drying is not only effective at preserving thermolabile compounds but may also improve carotenoid recovery during subsequent extraction and quantification. However, its practical application may be limited by long processing times and high operational costs (Sagar & Kumar, 2010).



<Figure. 1> Chemical structures of lutein and zeaxanthin (L/Z)

3.2 Physicochemical characteristics analysis

3.2.1 Solubility

Solubility is a critical property of food powders, as poor solubility can lead to processing challenges and economic losses (Sharma et al., 2012). As shown in <Table 2>, the solubility of spray-dried powder in water (0.17 □ 0.00 %) is significantly higher than that of freeze-dried powder (0.09 □ 0.00 %). This difference could be attributed to variations in beads size produced by the spray-drying process and the porous microstructure of the resulting powders. The enhanced solubility of spray-dried powder is likely due to its increased specific surface area, which facilitates improved interaction with water compared to freeze-dried powder (Nandiyanto et al.,2019). Conversely, the lower solubility of freeze-dried samples may be explained by the prolonged processing time required for freeze-drying, during which moisture removal occurs at a markedly slower rate than in the rapid evaporation process of spray-drying (Jadhav et al., 2024). In addition, Cai and Croke (2000) demonstrated that freeze-dried powders exhibit higher hygroscopicity than spray-dried powders. As a result, freeze-dried powders readily absorb moisture from the surrounding environment, leading to surface wetting and lump formation, which further reduces their solubility.

<Table 2> Comparison of solubility, WHC/OHC, EAI, and ESI among different egg yolk powders

	Solubility (%)	WHC (g/g)	OHC (g/mL)	EAI (m^2/g)	ESI (min)
SD	0.17 ± 0.00^{a}	1.32 ± 0.09^{a}	1.66 ± 0.21^{d}	3.85 ± 0.05^{ab}	167.84 ± 25.49^{c}
FD	0.09 ± 0.00^{b}	1.40 ± 0.07^{a}	2.03 ± 0.07^{cd}	4.00 ± 0.07^{a}	497.64 ± 30.02^{a}
SDE	0.18 ± 0.03^{a}	0.99 ± 0.03^{b}	2.26 ± 0.23^{b}	3.72 ± 0.07^{bc}	321.81 ± 11.36^{b}
FDE	0.11 ± 0.05^{b}	1.11 ± 0.03^{b}	3.34 ± 0.23^{a}	3.60 ± 0.10^{c}	493.92 ± 68.46^{a}

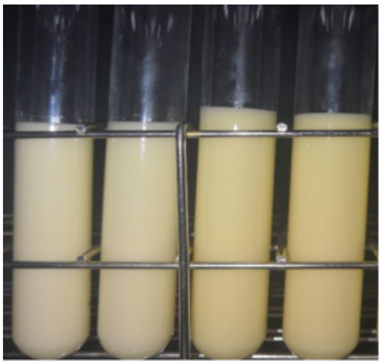
3.2.2 Water/Oil holding capacity (WHC/OHC)

The WHC and OHC values of all egg yolk powder are presented in <Table 2>. Among the four groups, the SD and FD samples exhibited significantly higher WHC than the L/Z enriched FDE and SDE. However, no direct correlation was observed was observed across the individual samples ($p > 0.05$), indicating that the drying method does not influence the WHC of the powder. The lower WHC observed in the L/Z-enriched group compared to the unenriched group can be attributed to the lipophilic nature of lutein, a carotenoid that has limited affinity for water, thereby reducing water-binding capacity (Kim & Shin, 2022).

OHC is a critical factor influencing emulsification ability, a desirable attribute for proteins and protein-based products such as mayonnaise (Sai-Ut et al., 2009). The OHC of freeze-dried samples was significantly higher than that of spray-dried samples ($p < 0.05$). Among the control groups, FD showed significantly greater OHC compared to SD. In the L/Z-enriched groups, FDE demonstrated the highest OHC, followed by SDE. This trend could be attributed to the loose structure of freeze-dried samples, which enhances the protein-lipid interaction and the facilitates the incorporation of hydrophobic fractions, resulting in improved oil retention (Mirhosseini & Amid, 2013).

3.2.3 Emulsifying activity index (EAI) and emulsion stability index (ESI)

As shown in <Table 2>, <Figuer 3>, EAI for SD and FD were 3.85±0.05 m^2/g and 4.00±0.07 m^2/g, respectively, indicating that the drying methods had no significant on the EAI of egg yolk powder. L/Z as fat-soluble carotenoids with low water compatibility, are typically incorporated into water-based systems through emulsions. The slightly lower EAI observed in FDE compared to FD can be attributed to the high L/Z content, which is challenging to emulsify and prone to instability post-emulsification. Similarly, the EAI of SD and SDE did not differ significantly (3.85 and 3.72 m^2/g, respectively) even with the added wall material glucose. Egg yolk is widely used as an emulsifying agent in a variety of food products, such as mayonnaise, salad dressings and sauces (Ma & Boye, 2013). Our findings demonstrate that enriching of egg yolk with L/Z does not affect its emulsification properties, indicating that L/Z-enriched egg yolk can be effectively used in the food and pharmaceutical industries.



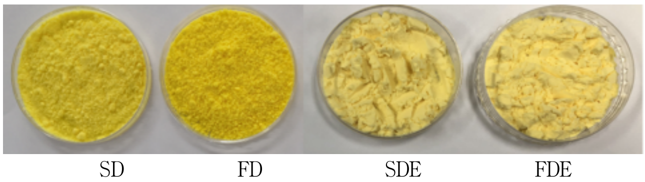
<Figure 3> Mayonnaises prepared using SD (spray-dried), FD (freeze-dried), SDE (spary-drying-enriched), and FDE (freeze-drying-enriched egg yolk powders) egg yolk powders

On the contrary, the drying methods revealed a significant impact on the ESI. Freeze-dried samples exhibited significantly higher ESI compared to spray-dried samples ($p < 0.05$), indicating greater interfacial stability and the ability to form strong interfacial membranes, thereby enhancing emulsion stability (Mundi & Aluko, 2012). These differences can be attributed to variations in water removal efficiencies and chemical alterations induced by the drying processes. Spray-drying involves rapid water removal through continuous spraying, mixing, and drying, leading to structural shrinkage and potential intermolecular interactions such as hydrogen bonding (Anwar & Kunz, 2011). In contrast, freeze-drying removes water under frozen conditions, facilitating the formation of thermally stable protein conjugates that improve oil emulsification and enhance emulsion interfacial area formation (Ratti, 2008).

In L/Z-enriched spray-dried powders, the ESI of SDE was significantly higher than that of SD, which could be due to differences in the carriers used. Arabic gum is widely used in spray-drying due to its excellent emulsifying capacity, which are attributed to its negatively charged molecular structure, abundant hydroxyl and carboxyl groups, and its ability to stabilize active substances and improve particle flow properties (Golkar et al., 2018). Arabic gum, has been reported allowing hydrophobic polypeptide chains to adsorb onto oil droplet surfaces while hydrophilic carbohydrate blocks extend outward, creating a steric barrier that prevents droplet agglomeration and coalescence (Guadarrama-Lezama et al., 2012). Additionally, maltodextrin, another commonly used carrier in spray-drying, offers benefits such as low hygroscopicity, minimal agglomeration, high solubility in cold water and low cost-effectiveness (Matioli & Rodriguez-Amaya, 2002). Maltodextrin also provides oxidative protection, depending on its dextrose equivalent. Overall, it can be inferred that despite the low emulsifying ability of SDE, it can be effectively used in the food industries by combining it with Arabic gum or other carriers that possess superior emulsifying properties (Costa et al., 2015).

3.3 Color determinations of yolk powder and mayonnaise

The representative samples of SD, FD, SDE, and FDE powders are shown in <Figure 2> Color values were measured using the $L^*a^*b^*$, where L^* indicates lightness, a^* represents red-green intensity (positive values indicate redness), and b^* corresponds to yellow-blue intensity (positive values indicate yellowness) (Pathare et al., 2013).



<Figure 2> Visual appearance of SD (spray-dried), FD (freeze-dried), SDE (spary-drying-enriched), and FDE (freeze-drying-enriched) egg yolk powders

The L^*, a^*, b^* values of the samples are presented in <Table 3>. Between the two control groups of yolk powder, SD showed higher L^* values as well as lower a^* and b^* values, indicating a lighter colorcompared to FD. This difference is likely due to the high temperatures employed during spray-drying, which may degrade pigments and result in reduced color intensity (Jiménez-González & Guerrero-Beltrán, 2021).These findings are further supported by the increased color values observed in SDE samples compared toSD samples. Similarly, FDE samples displayed lower L*and a*values but higher b*values than FD. These results suggest that the enhanced yolk color intensity in SDE and FDE samples can be attributable to the increased L/Z content achieved through dietary supplementation of hens with microalgae.

<Table 3> Chroma values of different egg yolk powder and mayonnaise samples

		L^*	a^*	b^*
Powder	SD	52.95 ± 0.62^{a}	-1.42 ± 0.16^{c}	17.00 ± 0.45^{c}
	FD	51.08 ± 0.67^{b}	-0.88 ± 0.06^{b}	17.94 ± 0.64^{b}
	SDE	51.11 ± 0.12^{b}	-0.91 ± 0.09^{b}	18.53 ± 0.25^{ab}
	FDE	48.37 ± 0.11^{c}	-0.13 ± 0.03^{a}	19.28 ± 0.52^{a}
Mayonnaise	SD	53.12 ± 1.01^{a}	-2.94 ± 0.06^{c}	8.32 ± 0.07^{c}
	FD	52.34 ± 0.41^{ab}	-2.32 ± 0.01^{bc}	8.64 ± 0.06^{c}
	SDE	51.24 ± 1.05^{b}	-1.86 ± 0.38^{ab}	15.80 ± 1.47^{a}
	FDE	49.20 ± 0.64^{c}	-1.57 ± 0.14^{a}	15.82 ± 0.53^{a}

A similar trend was observed in the mayonnaise samples. The L^* values of SD- and FD-based mayonnaise were higher than those of L/Z-enriched formulations. The darker appearance of enriched samples is expected due to the presence of lutein and zeaxanthin, which contribute to a more intense yellow-orange hue (Xu et al., 2022). Similarly, enriched samples exhibit higher b^* values (SDE: 15.80, FDE: 15.82) compared to unenriched ones (SD: 8.32, FD: 8.64). The significant increase in b^* values in the enriched mayonnaise formulations suggests that the pigments are well incorporated into the emulsion system, resulting in intensified yellow coloration.

Color is an important attribute in food products, significantly influencing consumer preferences as it serves as an indicator of freshness, quality and overall appeal. Moreover, it is also a key contributor to decide the taste acceptability of a product (Maskan, 2001). The color intensity of egg yolk, in particular, plays a critical role in consumer perception of freshness and quality. Deeper, more intense yolk color is generally preferred, as it is associated with better nutrition and natural feeding practices (Karadas et al., 2006). Color acceptability has also been shown to influence overall liking and purchase intent in mayonnaise and other egg-based products (Berkhoff et al., 2020). Consequently, producing egg yolk powders with consistent quality and intensified coloration is a priority for mayonnaise manufacturers to meet consumer demands and preferences.

3.4 Sensory evaluation

To ensure the reliability of the data, panelists were given brief training prior to evaluation, including clear definitions and evaluation guidance for each attribute. Aroma was assessed based on the intensity and pleasantness of the smell, flavor referred to the perceived taste and aftertaste, and stickiness was defined as the degree of tackiness felt on the tongue and palate. A 9-point hedonic scale was used <Table 4>.

Given the high fat content of mayonnaise samples, panelists were instructed to cleanse their palate not only with water but also with plain unsalted crackers between samples to eliminate residual oil and minimize carryover effects. Samples were presented in randomized order to reduce bias. Among the four sample groups, SDE and FDE (L/Z-enriched powders) exhibited the highest color scores, indicating that lutein and zeaxanthin contribute to a more intense yellow-orange hue, enhancing the visual appeal of mayonnaise. These samples also received the highest taste scores, suggesting that enrichment improves the overall flavor profile. Additionally, their flavor scores (SDE: 6.00, FDE: 5.65) were higher than those of the unenriched samples, confirming the positive impact of carotenoid enrichment on flavor perception. In terms of stickiness, SDE (6.10) and FDE (4.80) exhibited higher scores than their unenriched counterparts, implying that lutein and zeaxanthin may influence textural properties, potentially by enhancing emulsion stability (Jeong et al., 2021). The overall acceptability scores were also the highest for SDE (6.30) and FDE (5.85), indicating that L/Z enrichment improves the sensory quality of mayonnaise.

These results are consistent with the instrumental color measurements presented in Table 3. SDE and FDE mayonnaise exhibited significantly higher b* values (SDE: 15.80, FDE: 15.82), indicating greater yellowness. The higher b* values of SDE and FDE confirm the deeper yellow hue observed by the panelists, aligning with their higher color and acceptability scores. These findings are also in line with previous studies reporting that consumers tend to prefer egg-based products with a deeper yellow color, perceiving them as more natural and nutritious (Karadas et al., 2006; Amagloh et al., 2024). Therefore, L/Z-enriched mayonnaise developed in this study may offer enhanced appeal and potential for consumer acceptance in the market. FD samples received a higher color score than SD (spray-dried yolk powder), suggesting that freeze-drying better preserves natural yolk pigments, as demonstrated in Table 1. The flavor score of FD (5.20) was also higher than that of SD (4.10), implying that freeze-drying helps retain desirable flavor compounds more effectively than spray-drying. The stickiness score of FD (4.40) was slightly higher than SD (4.05), further supporting the idea that freeze-drying maintains better textural properties. SD samples exhibited the lowest scores in most sensory attributes, particularly in color, flavor, and overall acceptability. The lower flavor score (4.10) suggests that spray-drying may degrade flavor compounds to a greater extent than freeze-drying. However, aroma scores were relatively similar across all samples, indicating that neither drying method nor L/Z enrichment had a significant impact on aroma perception (Wang et al., 2023).

Overall, L/Z-enriched yolk powders (SDE, FDE) demonstrated superior sensory qualities in color, taste, and overall acceptability, making them preferable choices for food formulations. Additionally, freeze-drying emerged as the superior drying method for preserving the sensory attributes of egg yolk. These findings suggest that incorporating enriched egg yolk powders could enhance consumer acceptance and marketability of mayonnaise products.

<Table 4> Sensory evaluation of mayonnaise made with yolk powder

	Color	Aroma	Taste	Stickiness	Flavor	Overall
SD	4.95 ± 0.31^{b}	5.05 ± 0.24^{ab}	4.35 ± 0.48^{c}	4.05 ± 1.23^{c}	4.10 ± 1.37^{b}	4.40 ± 0.24^{b}
FD	6.00 ± 0.13^{ab}	4.95 ± 0.52^{b}	5.10 ± 0.26^{bc}	4.40 ± 0.13^{c}	5.20 ± 1.36^{a}	5.15 ± 0.76^{ab}
SDE	6.45 ± 1.76^{a}	6.05 ± 1.76^{a}	6.30 ± 1.76^{a}	6.10 ± 1.76^{a}	6.00 ± 1.76^{a}	6.30 ± 1.76^{a}
FDE	6.40 ± 1.76^{a}	6.00 ± 1.76^{a}	5.65 ± 1.76^{ab}	4.80 ± 1.76^{bc}	5.65 ± 1.76^{a}	5.85 ± 1.76^{a}

All values are expressed as a mean ± SD of triplicate estimations. ^{a,b,c}Means within columns with different letters are significantly different ($p < 0.05$).

IV. Conclusion

This study demonstrated the efficacy of *Dunaliella tertiolecta* mp3 in enriching egg yolks with lutein and zeaxanthin (L/Z), thereby enhancing the color intensity of egg yolk powders and mayonnaise. The L/Z content in yolk was well-preserved during the freeze-drying but showed a notable reduction in spary-drying due to the high-temperature involved. Overall, the findings suggest that L/Z-enriched dried egg yolk powder could be used as an emulsifying agent in food products such as mayonnaise, offering both functional and sensory benefits. However, the inherent instability of L/Z warrants further research to develop strategies for improving their chemical stability during storage.

References

Abdel-Aal, E.-S. M., Akhtar, H., Chambers, J. R., & Zaheer, K. (2017). Lutein and Zeaxanthin Carotenoids in Eggs. In *Egg Innovations and Strategies for Improvements* (pp. 199-206). Elsevier.

Amagloh, F. K., et al. (2024). *Yellowness of egg yolks influences consumer preference for eggs in Ghana.* International Food Research Journal, 31(2), 486-495

Anwar, S. H., & Kunz, B. (2011). The influence of drying methods on the stabilization of fish oil microcapsules: Comparison of spray granulation, spray drying, and freeze drying. *Journal of food engineering*, *105*(2), 367-378.

Asghar, A., & Abbas, M. (2012). Dried egg powder utilization, a new frontier in bakery products. *Agriculture and Biology Journal of North America*, *3*(13), 493-505.

Baek, K., Yu, J., Jeong, J., Sim, S. J., Bae, S., & Jin, E. (2018). Photoautotrophic production of macular pigment in a Chlamydomonas reinhardtii strain generated by using DNA-free CRISPR-Cas9 RNP-mediated mutagenesis. *Biotechnology and bioengineering*, *115*(3), 719-728.

Ben-Amotz, A. (1980). Glycerol production in the alga Dunaliella. *Biochemical and Photosynthetic aspects of energy production*, 191-208.

Berkhoff, J., Alvarado-Gilis, C., Keim, J. P., Alcalde, J. A., Vargas-Bello-Pérez, E., & Gandarillas, M. (2020). Consumer preferences and sensory characteristics of eggs from family farms. *Poultry science*, *99*(11), 6239-6246.

Bernstein, P. S., Li, B., Vachali, P. P., Gorusupudi, A., Shyam, R., Henriksen, B. S., & Nolan, J. M. (2016). Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Progress in retinal and eye research*, *50*, 34-66.

Cai, Y.-Z., & Corke, H. (2000). Production and properties of spray-dried Amaranthus betacyanin pigments. *Journal of Food Science*, *65*(7), 1248-1252.

Chhabra, N., Arora, M., Garg, D., & Samota, M. K. (2024). Spray freeze drying-a synergistic drying technology and its applications in the food industry to preserve bioactive compounds. *Food Control*, *155*, 110099.

Chung, H. Y., Rasmussen, H. M., & Johnson, E. J. (2004). Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. *The Journal of nutrition*, *134*(8), 1887-1893.

Costa, S. S., Machado, B. A. S., Martin, A. R., Bagnara, F., Ragadalli, S. A., & Alves, A. R. C. (2015). Drying by spray drying in the food industry: micro-encapsulation, process parameters and main carriers used. *African Journal of Food Science*, *9*(9), 462-470.

Fernández-Sevilla, J. M., Fernández, F. A., & Grima, E. M. (2010). Biotechnological production of lutein and its applications. *Applied microbiology and biotechnology*, *86*(1), 27-40.

Franke, K., & Kießling, M. (2002). Influence of spray drying conditions on functionality of dried whole egg. *Journal of the Science of Food and Agriculture*, *82*(15), 1837-1841.

Golkar, A., Taghavi, S. M., & Aghili Dehnavi, F. (2018). The emulsifying properties of Persian gum (Amygdalus scoparia Spach) as compared with gum Arabic. *International journal of food properties*, *21*(1), 416-436.

Guadarrama-Lezama, A. Y., Dorantes-Alvarez, L., Jaramillo-Flores, M. E., Pérez-Alonso, C., Niranjan, K., Gutiérrez-López, G. F., & Alamilla-Beltrán, L. (2012). Preparation and characterization of non-aqueous extracts from chilli (Capsicum annuum L.) and their microencapsulates obtained by spray-drying. *Journal of food engineering*, *112*(1-2), 29-37.

Hernández-García, S., Salazar-Montoya, J. A., & Totosaus, A. (2016). Emulsifying properties of food proteins conjugated with glucose or lactose by two methods (spray-drying or freeze-drying). *International journal of food properties*, *19*(3), 526-536.

Jadhav, K., Kole, E., Singh, R., Rout, S. K., Verma, R. K., Chatterjee, A., Naik, J. (2024). A critical review on developments in drying technologies for enhanced stability and bioavailability of pharmaceuticals. *Drying technology*, 1-27.

Jeon, J.-Y., Kim, K.-E., Im, H.-J., Oh, S.-T., Lim, S.-U., Kwon, H.-S., Kang, C.-W. (2012). The production of lutein-enriched eggs with dietary Chlorella. *Food Science of Animal Resources*, *32*(1), 13-17.

Jeong, S.-J., Kim, S., Echeverria-Jaramillo, E., & Shin, W.-S. (2021). Effect of the emulsifier type on the physicochemical stability and in vitro digestibility of a lutein/zeaxanthin-enriched emulsion. *Food Science and Biotechnology*, *30*(12), 1509-1518.

Jesús, M. N. d., Zanqui, A. B., Valderrama, P., Tanamati, A., Maruyama, S. A., Souza, N. E. d., & Matsushita, M. (2013). Sensory and physico-chemical characteristics of desserts prepared with egg products processed by freeze and spray drying. *Food Science and Technology*, *33*(3), 549-554.

Jiménez-González, O., & Guerrero-Beltrán, J. Á. (2021). Extraction, microencapsulation, color properties, and experimental design of natural pigments obtained by spray drying. *Food Engineering Reviews*, *13*(4), 769-811.

Junghans, A., Sies, H., & Stahl, W. (2001). Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Archives of Biochemistry and Biophysics*, *391*(2), 160-164.

Karadas, F., Grammenidis, E., Surai, P. F., Acamovic, T., & Sparks, N. H. C. (2006). Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. *British poultry science*, *47*(5), 561-566.

Kim, M.-J., & Shin, W.-S. (2022). Stability of zeaxanthin/lutein in yolk oil obtained from microalgae-supplemented egg under various storage conditions. *Lwt*, *155*, 112899.

Kim, M., Ahn, J., Jeon, H., & Jin, E. (2017). Development of a Dunaliella tertiolecta strain with increased zeaxanthin content using random mutagenesis. *Marine drugs*, *15*(6), 189.

Kishimoto, Y., Taguchi, C., Saita, E., Suzuki-Sugihara, N., Nishiyama, H., Wang, W., Kondo, K. (2017). Additional consumption of one egg per day increases serum lutein plus zeaxanthin concentration and lowers oxidized low-density lipoprotein in moderately hypercholesterolemic males. *Food Research International*, *99*, 944-949.

Kishk, Y., & Elsheshetawy, H. E. (2013). Effect of ginger powder on the mayonnaise oxidative stability, rheological measurements, and sensory characteristics. *Annals of Agricultural Sciences*, *58*(2), 213-220.

Kurniawan, J. M., Yusuf, M. M., Azmi, S. S., Salim, K. P., Prihastyanti, M. N. U., Indrawati, R., Brotosudarmo, T. H. P. (2019). Effect of drying treatments on the contents of lutein and zeaxanthin in orange-and yellow-cultivars of marigold flower and its application for lutein ester encapsulation. IOP Conference Series: Materials Science and Engineering,

Landrum, J. T., & Bone, R. A. (2001). Lutein, zeaxanthin, and the macular pigment. *Archives of biochemistry and biophysics*, *385*(1), 28-40.

Ma, L., Liu, R., Du, J. H., Liu, T., Wu, S. S., & Liu, X. H. (2016). Lutein, zeaxanthin and meso-zeaxanthin supplementation associated with macular pigment optical density. *Nutrients*, *8*(7), 426.

Ma, Z., & Boye, J. I. (2013). Advances in the design and production of reduced-fat and reduced-cholesterol salad dressing and mayonnaise: a review. *Food and Bioprocess Technology*, *6*(3), 648-670.

Marante, T., Viegas, C., Duarte, I., Macedo, A. S., & Fonte, P. (2020). An overview on spray-drying of protein-loaded polymeric nanoparticles for dry powder inhalation. *Pharmaceutics*, *12*(11), 1032.

Maskan, M. (2001). Kinetics of colour change of kiwifruits during hot air and microwave drying. *Journal of food engineering*, *48*(2), 169-175.

Matioli, G., & Rodriguez-Amaya, D. (2002). Licopeno encapsulado em goma arábica e maltodextrina: Estudo da estabilidade. *Braz. J. Food Technol.*, *5*, 197-203.

Meera, V., Ved, S., & Vino, S. A. (2016). Development of value added Probiotic freeze-dried papaya juice powder. *Journal of Foodservice*, *3*, 38-48.

Miranda, J. M., Anton, X., Redondo-Valbuena, C., Roca-Saavedra, P., Rodriguez, J. A., Lamas, A., Cepeda, A. (2015). Egg and egg-derived foods: effects on human health and use as functional foods. *Nutrients*, *7*(1), 706-729.

Mirhosseini, H., & Amid, B. T. (2013). Effect of different drying techniques on flowability characteristics and chemical properties of natural carbohydrate-protein Gum from durian fruit seed. *Chemistry Central Journal*, *7*(1), 1.

Motta-Romero, H., Zhang, Z., Tien Nguyen, A., Schlegel, V., & Zhang, Y. (2017). Isolation of egg yolk granules as low-cholesterol emulsifying agent in mayonnaise. *Journal of food science*, *82*(7), 1588-1593.

Mundi, S., & Aluko, R. (2012). Physicochemical and functional properties of kidney bean albumin and globulin protein fractions. *Food Research International*, *48*(1), 299-306.

Nandiyanto, A. B. D., Ogi, T., Wang, W.-N., Gradon, L., & Okuyama, K. (2019). Template-assisted spray-drying method for the fabrication of porous particles with tunable structures. *Advanced Powder Technology*, *30*(12), 2908-2924.

Pathare, P. B., Opara, U. L., & Al-Said, F. A. J. (2013). Colour measurement and analysis in fresh and processed foods: A review. *Food and bioprocess technology*, *6*, 36-60.

Ratti, C. (2008). Freeze and vacuum drying of foods. *Drying technologies in food processing*, 225-251.

Roberts, J. E., & Dennison, J. (2015). The photobiology of lutein and zeaxanthin in the eye. *Journal of ophthalmology,2015*.

Sagar, V., & Kumar, P. S. (2010). Recent advances in drying and dehydration of fruits and vegetables: a review. *Journal of food science and technology*, *47*(1), 15-26.

Sai-Ut, S., Ketnawa, S., Chaiwut, P., & Rawdkuen, S. (2009). Biochemical and functional properties of proteins from red kidney, navy and adzuki beans. *Asian Journal of Food and Agro-Industry*, *2*(4), 493-504.

Samborska, K., Jedliñska, A., Wiktor, A., Derewiaka, D., Wo³osiak, R., Matwijczuk, A., B³a¿owski, £. (2019). The effect of low-temperature spray drying with dehumidified air on phenolic compounds, antioxidant activity, and aroma compounds of rapeseed honey powders. *Food and Bioprocess Technology*, *12*, 919-932.

Sharma, A., Jana, A. H., & Chavan, R. S. (2012). Functionality of milk powders and milk-based powders for end use applications—a review. *Comprehensive Reviews in Food Science and Food Safety*, *11*(5), 518-528.

Sogi, D. S., Siddiq, M., & Dolan, K. D. (2015). Total phenolics, carotenoids and antioxidant properties of Tommy Atkin mango cubes as affected by drying techniques. *LWT-Food Science and Technology*, *62*(1), 564-568.

Sui, X., Kiser, P. D., Che, T., Carey, P. R., Golczak, M., Shi, W., Palczewski, K. (2014). Analysis of carotenoid isomerase activity in a prototypical carotenoid cleavage enzyme, apocarotenoid oxygenase (ACO). *Journal of Biological Chemistry*, *289*(18), 12286-12299.

Tsiaka, T., Lantzouraki, D. Z., Siapi, E., Sinanoglou, V. J., Heropoulos, G. A., Calokerinos, A. C., & Zoumpoulakis, P. (2018). Macular carotenoids in lipid food matrices: DOE-based high energy extraction of egg yolk xanthophylls and quantification through a validated APCI (+) LC-MS/MS method. *Journal of Chromatography B*, *1096*, 160-171.

Wang, Z., Nie, T., Zhang, H., Wang, W., Chen, H., Wang, S., & Sun, B. (2023). Correlation analysis between volatile compounds and quality attributes in pork tenderloin in response to different stir-frying processes. *Foods*, *12*(23), 4299.

Wenzel, M., Seuss-Baum, I., & Schlich, E. (2010). Influence of pasteurization, spray-and freeze-drying, and storage on the carotenoid content in egg yolk. *Journal of agricultural and food chemistry*, *58*(3), 1726-1731.

Xu, L., Echeverria-Jaramillo, E., & Shin, W.-S. (2022). Physicochemical properties of muffins prepared with lutein & zeaxanthin-enriched egg yolk powder. *Lwt*, *156*, 113017.

Zhang, L.-h., & Li, S.-f. (2009). Effects of micronization on properties of Chaenomeles sinensis (Thouin) Koehne fruit powder. *Innovative food science & emerging technologies*, *10*(4), 633-637.