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Effect of freezing under high voltage electrostatic fields on physical characteristics of chicken breast

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Abstract

Freezing is one of the most common methods for preserving food as it is an effective way to extend shelf life and preserve flavor and color. Currently, the high voltage electrostatic field (HVEF) has been studied to assist freezing meats such as lamb and pork. The results showed that the drip loss decreased, and textures were closed to unfrozen meat. However, many works had studied the effect of HVEF assisted freezing on texture of thawed small meat samples (around 1 – 15 g), and there was little information on texture of cooked products. The objective of this research is to study the physical characteristics of cooked chicken breast, with higher weight (38 ± 2 g.), frozen under different HVEF intensities. The chicken breasts were cut into 2 cm thickness and sealed in plastic bag (LDPE). The HVEF intensities (0, 1.25, 1.875, 2.5 and 3.125 kV/cm) were applied with freezing temperature at -20°C for 5 h. Frozen samples were kept at -40°C until they were analyzed. Then the samples were thawed at ambient temperature and cooked at 95-100°C. The drip loss and cooking loss of HVEF treatment decreased when they were compared to conventional freezing. The moisture content of cooked products was higher than those of products without HVEF. For texture, the hardness and chewiness of cooked products were decreased statistically significant difference from non-HVEF treatment, but the springiness and cohesiveness were not significantly different.

Keywords: Chicken breast, freezing, high voltage electrostatic field, texture

1. Introduction

Freezing is one of the ancient and most preferred process, used for preservation in meat products. In the frozen states, enzyme activity and microbial growth are inhibited (Hu *et al.,* 2022). Compared to the other processes, it has lower effect on deterioration of food properties such as color, flavor, nutrition (Delgado and Sun, 2001; Reid, 1993). However, during freezing process, ice crystal formations damage meat cells leading to drip loss, cooking loss and texture change. Therefore, many researchers have studied new emerging technologies to assist freezing process for instance ultrasound (Cheng *et al.,* 2015; Kiani *et al.,* 2011), high pressure (Chevalier *et al.,* 2000; Su *et al.,* 2014), magnetic electric field (Abedini *et al.,* 2011; Mohanty, 2001), radio frequency (Anese *et al.,* 2012) and high voltage electrostatic field (HVEF). The most promising technology to apply frozen food industry is HVEF because of its cost effectiveness and easy to integrate with commercial machines (Dalvi-Isfahan *et al.,* 2016; Le-Bail *et al.,* 2011; Wang *et al.,* 2019; Xanthakis *et al.,* 2013). High voltage electrostatic field can be produced by applying high voltage electricity to parallel electrodes. The lines of electrostatic flux are in between oppositely charged electrodes. By increasing the electrostatic fields, nucleation temperatures are shifted to higher values (Jia *et al.,* 2017; Orlowska *et al.,* 2009) leading to reduce freezing times, increase freezing rates, and create abundant small ice crystals (Wang *et al.,* 2019).

For recent years, HVEF has been studied to improve the qualities of frozen meats such as pork (Xanthakis *et al.,* 2013), lamb (Dalvi-Isfahan *et al.,* 2016; Dalvi-Isfahan *et al.,* 2018), shrimp (Liu *et al.,* 2022) on drip loss, color, texture of thawed samples etc. However, these works have been done with a small piece of meat samples (1.06 ± 0.04 g. for pork, 2.3 ± 0.2 g for lamb, 14.7 ± 1.9 g for shrimp). Moreover, there are still limited results on cooking loss and texture of cooked samples. Therefore, the aim of this study was to investigate drip loss, cooking loss and texture of cooked chicken breasts, in which the chicken breasts (around 38 g.) are frozen under HVEF.

2. Materials and Methods

2.1 Materials

Chicken breasts were bought from Ya-Mo market (Nakornratchasima, Thailand) on the slaughtered day and packed in the ice boxes in the morning. The breasts were cut into 2 cm thickness, weighed for 38 ± 2 g. per piece and sealed in low density polyethylene (LDPE) vacuum bag. The length and width of samples depended on the shape of breasts which were not fixed. All samples were cut and frozen under each condition on the same day. The moisture content of samples was measured by using moisture analyzer (Model 350-8860/K HA 300, Precisa Instruments AG, Dietikon, Switzerland).

2.2 Freezing under high voltage electrostatic fields

2.1.1 Freezing time

The freezing time was investigated by using thermocouples (Benetech, Model GM1312, Shenzhen, China). The thermocouples inserted to the center of chicken breasts and temperatures were observed (without HVEF). The temperature of samples reduced from 8.7 and 10°C to lower than -10°C for 4.2 h. Therefore, in this experiment, the samples were frozen under different HVEF intensities in the freezer for 5 h.

2.2.2 High voltage electric field apparatus

Fig 1. showed the diagram of experimental set up. High voltage electrostatic field, HVEF, was generated from a direct power supply (CYXJ300AN, China). Electrodes were made from 304 stainless plates with dimensions of 20 × 25 cm placed in parallel. The distance between electrodes was 8 cm. The samples, contained in LDPE vacuum bags, were placed between electrodes and applied voltages at 10, 15, 20 and 25 kV (electrostatic field intensities of 1.25. 1.875, 2.5 and 3.125 kV/cm) inside the freezer (Sanyo, SF-C992 NG, Thailand) at -20°C for 5 h. The freezer dimensions were 105 × 65 × 87 cm. The control samples (0 kV/cm) were simultaneously frozen with all experimental treatments. Then the frozen samples were stored in freezer at -40°C until they were analyzed. Electrostatic field intensities (I_{HVEF}) were calculated by applied voltages (V) dividing with the distance between electrodes (D) following Equation (1).

$$I_{HVEF}\ (kV/cm) = (V\ [kV])/(D\ [cm.]) \qquad (1)$$

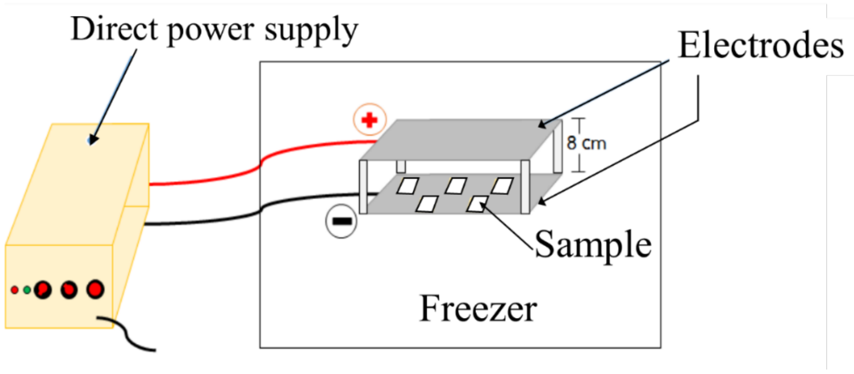

Fig 1 Diagram of the experimental set up

The samples of cut chicken breasts, 7 pieces, were arranged on the electrode as shown in fig 2.

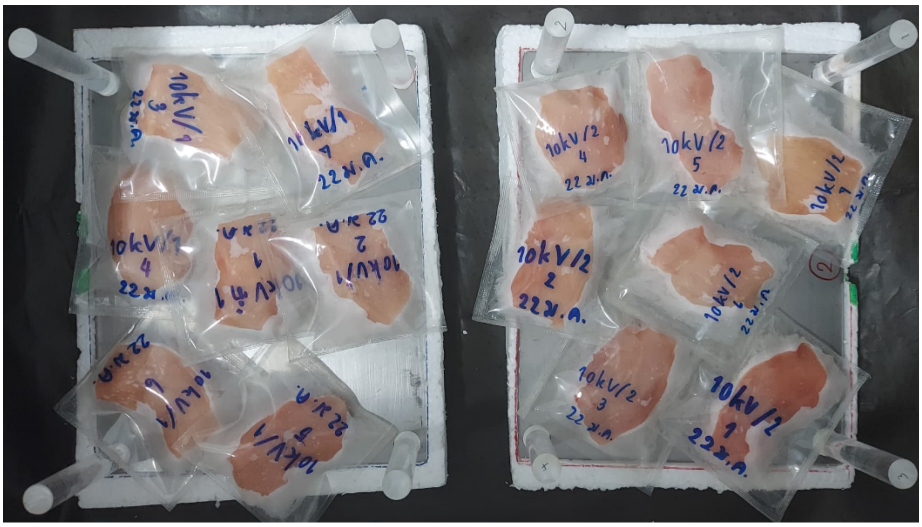

Fig 2 Arrangement of chicken breasts, with 2 cm. thickness and weighted for 38 ± 2 g in LDPE vacuum bag, on electrode.

2.2.3 High voltage measurement

High voltages, generated from a direct power supply (TZT, Model CYXJ300AN, Shenzhen, China), were measured by using voltage divider method. Using ohm's law, the resistors were connected in series circuit as shown in Fig 3. The voltage was divided by 10 resistors, in which R_1 – R_9 had resistance of 56 MΩ and R_{10} had resistance of 10 kΩ. The voltage drop across the tenth resistor ($V_{measured}$) was measured by using digital multimeter. Voltage of power supply (V_{in}) was calculated from Equation (2) (Irwin and Nelms, 2020).

$$V_{in} = V_{measured} \times \frac{(R_1 + R_2 + \ldots + R_{10})}{R_{10}} \qquad (2)$$

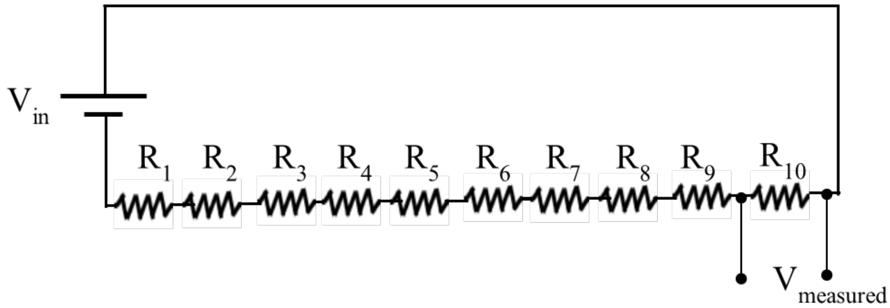

Fig 3 Schematic diagram of Voltage divider method.

2.3 Physical characteristics

2.3.1 Drip loss and cooking loss

Frozen samples were weighed (W_1) and then thawed at ambient temperature (24-26°C) before they were weighed (W_2). As thawing time at ambient temperature was reduced around 70% compared to thawing time at refrigerator temperature (Xu *et al.,* 2021). Furthermore, there were no significant differences in firmness (Xu *et al.*, 2021) and tenderness (Gomes *et al.*, 2021) of thawed frozen at ambient and refrigerator temperature. Drip loss percentage was calculated and expressed using Equation (3).

$$\%\ \text{Drip loss} = \frac{W_1 - W_2}{W_1} \times 100 \qquad (3)$$

The thawed samples were sealed in polyethylene bags and cooked in hot water at 95-100°C with water bath (Julabo, SW22, Thailand (until the temperature of center reached 75°C. Then the cooked samples were cooled in water at 25°C and then weighed cooked sample (W_3). Cooking loss percentage was determined and shown using Equation (4).

$$\%\ \text{Cooking loss} = \frac{W_2 - W_3}{W_2} \times 100 \qquad (4)$$

2.3.2 Texture measurement

After cooking, the samples were cut into cubic pieces (1.5 × 1.5 × 1.5 cm.) for texture analysis using texture profile analysis (TA-XT plus Texture Analyzer, UK) with probe P/36R. The parameters were set with the pretest speed of 2 mm/s, the test speed of 1 mm/s and the post-test speed of 5 mm/s and compressed samples to 0.6 cm. (Zhang *et al.*, 2020). The cutting samples of all treatments were measured in longitudinal and transverse fiber muscle directions. Hardness, springiness, cohesiveness, and chewiness were calculated.

2.4 Statistical analysis

A completely randomized design (CRD) was used to study the electrostatic field intensity factors of 0, 1.25. 1.875, 2.5 and 3.125 kV/cm. Three replications were performed for all treatments. SPSS software version 16 (SPSS Inc., IL, USA) was used for statistical analysis. Duncan's multiple range test was applied to compare the difference of means between each treatment at 95 %confidence interval.

3. Results and Discussion

3.1 Effect of HVEF assisted freezing on drip loss, cooking loss and moisture content of cooked chicken meats

In this study, all chicken breast samples had initial moisture contents around 70% wet basis with no statistically significant difference as shown in Table 1. The results in Table 1 also showed the effect of freezing chicken breasts under different high voltage electrostatic field (HVEF) intensities on drip loss, cooking loss percentages and moisture content of cooked chicken breasts.

By using HVEF intensities from 1.25 to 3.125 kV/cm assisted freezing chicken breasts, the drip loss was lower than that of freezing with non-HVEF conditions. But the drip loss had no significant difference with the different HVEF intensities. These results were similar to the works of Dalvi-Isfahan *et al.*, 2016; Dalvi-Isfahan *et al.*, 2018; Jia *et al.*, 2017. The cooking loss of the samples first decreased at lower HVEF intensities (1.25 and 1.875 kV/cm.) and then increased at higher HVEF intensities (2.5 and 3.25 kV/cm.). As the drip loss and cooking loss of HVEF treatments were lower than those of the control (0 kV/cm.), therefore the moistures of cooked meats treated with HVEF were higher than those of conventional freezing.

Table 1 % Initial moisture content, %drip loss, %cooking loss and %moisture content of cooked products of frozen chicken breasts under different high voltage electrostatic field intensities (I_{HVEF}).

I_{HVEF} (kV/cm)	% MC_{wb} of initial meats	% Drip loss	% Cooking loss	% MC_{wb} of cooked meats
0	70.62 ± 0.74^{a}	2.07 ± 0.62^{b}	21.15 ± 3.77^{bc}	65.67 ± 0.91^{a}
1.25	70.63 ± 0.08^{a}	1.40 ± 0.48^{a}	19.51 ± 3.37^{ab}	67.23 ± 0.57^{b}
1.875	69.98 ± 0.08^{a}	1.59 ± 0.66^{a}	17.87 ± 4.49^{a}	67.36 ± 0.17^{b}
2.5	70.09 ± 0.67^{a}	1.41 ± 0.62^{a}	22.78 ± 3.05^{c}	67.01 ± 0.39^{b}
3.125	70.75 ± 0.22^{a}	1.50 ± 0.68^{a}	23.35 ± 2.38^{c}	66.95 ± 0.16^{b}

Note: $^{a, b, c}$ Means ± Standard deviation of 3 pieces × 3 replicates (n = 9) were presented with different superscripts are significantly different (P<0.05) in the same column.

Generally, big ice crystals are formed during conventional freezing process, which caused cell tissue damage leading to drip loss during thawing (Orlowska *et al.*, 2009). Then the cell loses muscle fluid and texture changes (Morrison, 1993). On the other hand, the freeze-thaw process induces protein denaturation and aggregation with ice crystallization on solute concentrations, cold denaturation, and protein oxidation leading to loss of qualities (Amiri *et al.*, 2019; Lee *et al.*, 2022; Xia *et al.*, 2009). The freezing under HVEF process produces smaller and more uniform ice crystals than conventional process (Dalvi-Isfahan *et al.*, 2016, Jia *et al.*, 2017, Xanthakis *et al.*, 2013). Wei *et al.*, 2008 explained that water molecules tended to align along the direction of the electrostatic field and nucleation in water supercooled can be induced to raise the temperature of supercooling. Dalvi-Isfahan *et al.*, (2018) presented micrographs of frozen lamb meats with different HVEF intensities and non-HVEF, in which the ice crystal diameters of HVEF treatments are smaller. However, there are no significant differences among of the HVEF treatments. Dalvi-Isfahan *et al.*, (2017) have descripted the mechanism of ice crystal size reduction that HVEF reduces the entropy due to destruction of some hydrogen bonds and arranges water molecule in the direction of electric field, enhancing ice nucleation process. Then the hydrogen bonds are stronger, and the water structural clusters are changed. These might also be results of lower drip loss, cooking loss and higher moisture content due to the different size of ice crystals produced from HVEF and non-HVEF assistance.

3.2 Effect of HVEF assisted freezing on texture of cooked chicken meats in direction of longitudinal and transverse fiber muscles

Textural properties (hardness, springiness, cohesiveness, and chewiness) of the cooked samples, obtained from 4 pieces × 3 replicates × 4 HVEF intensities (*n*=48) for control (0 kV/cm) and 4 pieces × 3 replicates (*n*=12) for each HVEF intensity (1.25, 1.875, 2.5 and 3.125 kV/cm), were measured in longitudinal and transverse fiber muscle directions.

Table 2 Texture of cooked chicken breasts from freezing under HVEF process measured in direction of longitudinal fiber muscles.

I_{HVEF} (kV/cm)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (mJ)
0	32.24 ± 7.34^{b}	0.61 ± 0.06^{b}	0.37 ± 0.06^{a}	7.23 ± 2.49^{b}
1.25	24.63 ± 2.98^{a}	0.59 ± 0.06^{ab}	0.34 ± 0.03^{a}	5.04 ± 1.06^{a}
1.875	28.50 ± 3.81^{ab}	0.57 ± 0.04^{a}	0.35 ± 0.05^{a}	5.71 ± 1.44^{a}
2.5	31.90 ± 5.56^{b}	0.61 ± 0.04^{b}	0.38 ± 0.06^{a}	7.47 ± 2.24^{b}
3.125	27.17 ± 3.23^{a}	0.57 ± 0.05^{ab}	0.35 ± 0.06^{a}	5.40 ± 1.30^{a}

Note: $^{a, b}$ Means ± Standard deviation with different superscripts are significantly different ($P<0.05$) in the same column.

Table 2 showed the texture of cooked meats, measured in longitudinal fiber muscle direction, while the texture detected in direction of transverse fiber muscles were presented in Table 3. The results showed that the effect of HVEF on texture properties of both directions was a similar tendency. The cooked meats, frozen with HVEF, were more tender than the control, frozen with no HVEF.

Table 3 Texture of cooked chicken breasts from freezing under HVEF process in direction of transverse fiber muscles.

I_{HVEF} (kV/cm)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (mJ)
0	28.31 ± 5.07[b]	0.625 ± 0.08[a]	0.375 ± 0.04[a]	6.630 ± 1.73[bc]
1.25	23.11 ± 2.01[a]	0.655 ± 0.05[a]	0.373 ± 0.03[a]	5.657 ± 0.88[ab]
1.875	23.12 ± 2.47[a]	0.625 ± 0.04[a]	0.370 ± 0.03[a]	5.366 ± 1.03[a]
2.5	28.31 ± 5.77[b]	0.662 ± 0.08[a]	0.377 ± 0.05[a]	7.148 ± 2.33[a]
3.125	25.16 ± 4.57[a]	0.626 ± 0.06[a]	0.383 ± 0.04[a]	6.120 ± 1.81[abc]

Note: [a, b, c] Means ± Standard deviation with different superscripts are significantly different ($P<0.05$) in the same column.

By increasing HVEF intensities assisted freezing process, the changing trends in hardness and chewiness properties were quite similar. Their values of cooked chicken breasts, frozen with HVEF, were lower than those of non-HVEF in both longitudinal and transverse fiber directions. While the springiness and cohesiveness had no effect from the HVEF intensities, there were no significant differences from the control treatment for both directions. These results were in agreement with the reports of Jia *et al.* (2017). Jia *et al.* (2018) revealed that during freezing process, HVEF can reduce myosin denaturation while sarcoplasmic proteins and actin were less sensitive. Myofibrillar proteins including myosin are associated with water holding capacity, while myofibrillar proteins were also denatured during the thermal process (Bowker and Zhuang, 2015; Rahbari *et al.*, 2018; Zhang and Ding, 2020). Therefore, texture of frozen meat treated by HVEF had lower hardness and higher moisture content than non-HVEF because of smaller ice crystal size leading to less cell damage (Dalvi-Isfahan *et al.*, 2018; Jha *et al.*, 2017) and less protein denaturation (Jia *et al.*, 2017).

4. Conclusion

In this study, the freezing of chicken breast samples under HVEF system using 4 different intensities (1.25, 1.875, 2.5 and 3.125 kV/cm) was investigated. The results showed that freezing under all HVEF intensities significantly reduced drip loss and cooking loss. Texture properties, measured from both fiber muscle directions, changed in the same trends. There was no effect of HVEF intensities on springiness and cohesiveness of cooked samples, but HVEF, at intensity 1.25 kV/cm, affected to their hardness and chewiness. Therefore, freezing under HVEF could improve the physical characteristics of chicken breast.

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Food and Applied Bioscience Journal

Aging Mechanism and Anti-Aging Foods for the Elderly: A Review

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Abstract

Living and eating healthiness have been the topic of interest for the elderly and their caregivers. Functional foods with balanced nutrition and anti-aging properties were considered and researched continuously. This review aimed to summarize and discuss the aging process and functional foods from natural sources for the elderly using studies conducted between 1996 and 2023 that focus on their effectiveness to alleviate aging symptoms. Aging is a complex process with various mechanisms including oxidative stress, hormonal system, activity of the enzyme sirtuin, and intestinal microbiota. The aging process leads to many disorders in the elderly such as Alzheimer's, Parkinson's, and non-communicable diseases. Additionally, it was found that balanced nutrients and energy along with the consumption of natural antioxidants from vitamins, phytochemicals, and sirtuin activator foods were effective at slowing down aging and extending an individual's lifespan. Recently, innovative research into foods for the elderly has focused on their sensory perception and whether they consist of the necessary nutrients. Therefore, this review might support a future perspective product of the food and pharmaceutical industries because of the different requirements of the elderly in society.

Keywords: Elderly, Aging Process, Anti-aging Food, Sirtuin Activator Foods, Diet Pattern

1. Introduction

The interesting forecast data from 2025 to 2050 of He *et al.* (2016) illustrates that the total global population will grow by about 34%, while the older population was estimated to almost double to 1.6 billion in the same period. The American Hospital Association (2008) estimated that the expense of treating age-related disorders would increase proportionally according to their severity with many hospital admissions expected (Association, 2008; Garza, 2016). Recently, numerous tactics have been implemented in anti-aging healthcare including behavior modification, public health, medical technology, and the management of facilities in care homes and gained attention (Leung *et al.*, 2013). It is widely recommended that the elderly and their caregivers engage in anti-aging behavior modification, particularly regarding their daily diet and meals, due to the easily accessible and beneficial health advantages. Dietary sources of energy, minerals, micronutrients, antioxidants, and phytochemicals are essential for the promotion of elderly health and the prevention of diseases associated with aging.

Consequently, the purpose of this review was to illustrate the aging mechanism and associated alterations. Moreover, the sources of anti-aging foods for the elderly, including natural antioxidant and sirtuin activator foods, were collected and represented. Dietary guidelines for the elderly, including calorie restriction, a balanced intake of macro- and micronutrients, and an anti-aging food pyramid, were presented and discussed. Trends in the development of food for the elderly were also highlighted in this work.

2. Aging mechanism

Some of the mechanisms of aging discovered by numerous research in recent years have been discussed. Fig 1 provides an illustration of the aging mechanism discussed in this review. In this review, we investigated several aging mechanisms that may be implicated in human physiology, such as oxidative stress, the endocrine system, sirtuin (SIRT) enzymes, the immune system, gut microbiota, and gene modulation.

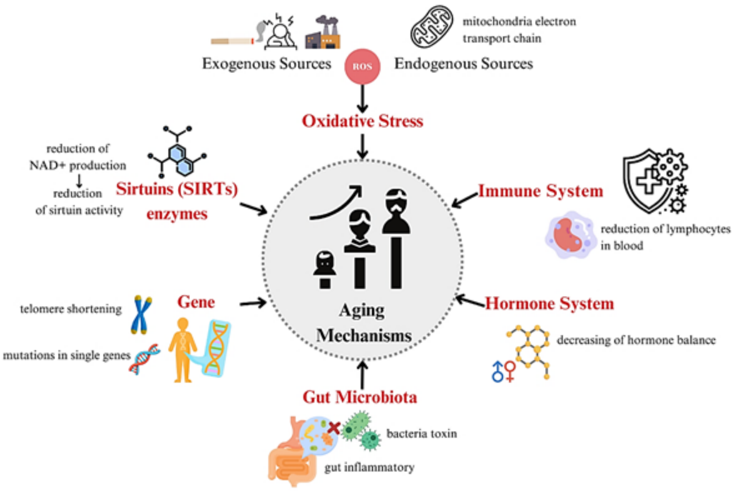

Fig 1 Mechanism of human aging.

2.1 Oxidative stress

Increasing oxidative stress is associated with the aging process, caused by an imbalance between the generation of free radicals and the body's ability to prevent them. The production of reactive oxygen species (ROS), also known as free radical derivatives of oxygen, results from the formation of potentially harmful byproducts. They are derived from both endogenous and exogenous sources (Phaniendra *et al.*, 2015). Beckman and Ames (1998) concluded that ROS is the cause of structural damage and that oxidative damage to macromolecules leads to age-related functional decreases.

2.2 Hormone system

According to the observation of Monzani *et al.* (2016), there was a possibility that there was a connection between the natural aging process and the regulation of hormones in the body. When the mechanism that controls sex hormones in men and women as part of ageing fails, it may contribute to changes in musculoskeletal and sexual dysfunction as well as long-term health disease concerns (El-Sakka Ahmed and Hassoba, 2006).

2.3 Sirtuins (SIRTs) enzymes

Sirtuin, the family of histone deacetylases (HDACs), role in the mechanism of the aging process includes scleroderma, brain aging, and neurodegenerative disorders (Wyman and Atamas, 2018). The level of these enzymes decreases during aging while their regulation relieves the symptoms of cellular senescence (Grabowska *et al.*, 2017). SIRTs can restore the balance of protein by reducing the accumulation of toxins, improving mitochondria function by reducing oxidative stress, and raising the level of nervous system function by elevating the transcription of learning and memory genes (Min *et al.*, 2013). Poulose and Raja (2015) summarized that humans encode seven sirtuin isoforms (SIRT1- SIRT7) with a variable intracellular distribution. Sirtuins have been linked to longevity, and evidence suggests that they have a role in a variety of diseases including cancer, diabetes, obesity, and neurodegenerative disorders. In addition, numerous studies have shown that the SIRT1-mediated deacetylation of targets has a substantial effect on mitochondrial function, apoptosis and inflammation. These processes and activities are critical for defining life span as well as the effect of an injury with reports indicating the relation of the latter to SIRT1 activity (Nakagawa and Guarente, 2011; Salminen *et al.*, 2008; Zhang *et al.*, 2010).

2.4 Immune system

Oliveira *et al.* (2010) discussed how immune system changes occur during the aging process. The changes due to aging are complicated and result in a decrease in certain immune response parameters, qualitative changes in response, and even the worsening of certain immunological processes. Nikolich-Žugich (2012) depicted how immune system aging affects the development of lymphocytes, originating within hematopoietic stem cells and their pluripotent progeny that will become lymphocytes.

2.5 Other aging mechanisms

Current research articles indicate the importance of the composition, diversity, and functional features of the intestinal microbiota in relation to aging-associated alterations (Vaiserman *et al.*, 2017). Age-related disorders in the gut microbiome are an important pathological state, and touch on neurodegeneration and cardiovascular disease (Friedland, 2015; Sanduzzi *et al.*, 2016). Some of the articles represent key findings pathways that support an epigenetic role in aging (Sen *et al.*, 2016). Recent studies have demonstrated a genetic involved to human aging and longevity.

Rodríguez-Rodero *et al.* (2011) reviewed that mutations in certain genes have been found to provide greater resistance to stress and a slower rate of damage accumulation, resulting in increased longevity. Zhang *et al.* (2023) presented an understanding of how mutations in single genes can significantly impact lifespan and provided an example of Eriksson *et al.* (2003) that demonstrated that Lamin A, a major constituent of the nuclear matrix, has mutations in exon 11 of the LMNA gene that can impair nuclear structure and function, resulting in premature aging. Additionally, Telomeres, which are DNA-protein complexes that cap the ends of linear DNA strands, serve to stabilize the chromosomes and prevent instability. There is a correlation between telomere shortening and the decline of somatic stem cells during the aging process (Collado *et al.*, 2007; O'sullivan and Karlsederl, 2010)

3. Aging-related diseases and symptoms

Aging is associated with wrinkles, decreased brain function, and an increased risk of chronic disease. Interestingly, sarcopenia can be a risk factor of dysphagia in the elderly (mean age 82.5 ± 8.4 years) because of a loss of skeletal muscle mass and decreased muscle strength which results in a decreased swallowing function (Maeda and Akagi, 2016). The memory functions of the elderly, mainly verbal such as digit span, word span, and story recall, normally decline with aging. Alzheimer's disease and Parkinson's disease are the most common causes of demen*tia.* Low testosterone level is relatively associated with an increased risk of worse cognitive function and Alzheimer's disease among elderly men (Lv *et al.*, 2016). Several changes in the cardiovascular system during the normal aging process, including changes in the structural components in the arteries, decreasing vascular elasticity, and abnormality of the endothelium of aged vessels, lead to specific cardiovascular diseases in the elderly. Moreover, some non-communicable diseases (NCDs) are risk factors for coronary heart disease such as diabetes, obesity, and hypertension (Jackson and Wenger, 2011). Metabolic syndrome in older people was largely (73.1%) associated with cardiovascular risk by considering the relationship between body mass index and cardiovascular disease (Dhana *et al.*, 2016).

Various researchers are driven to discover solutions to age-related diseases and symptoms, such as anti-aging drugs, herbal supplements, and artificial intelligence (AI) technology. This article concentrates on food and related topics as potential solutions. Nevertheless, there are limitations and important factors contributing to decreased appetite and food intake of elderly people such as physical, mental, swallowing and mastication difficulties. Physical functioning (e.g., activities of daily living, leisure and social activities, general physical activities, etc.) is essential for food security among elderly individuals, and thus resources should be provided to enable the acquisition, preparation, and consumption of food for those with physical impairments (Jackson *et al.*, 2019). In the meantime, Rusu *et al.* (2020) emphasized the importance of regularly assessing the overall nutritional status (e.g., body weight, lean body mass, etc.) and effectively communicating the need for nutrient supplementation.

Furthermore, a considerable amount of research has been investigating the correlation between the diverse backgrounds of the elderly and their food sources. The behavior of elderly individuals from different backgrounds, such as urban and rural areas, is a contributing factor to the successful implementation of food personalization. Gao *et al.* (2022) suggested that elderly people living in rural areas may improve their health by advocating intergenerational cohabitation, increasing their consumption of fruits and vegetables, and maintaining their intake of protein to achieve a balanced diet. Interestingly, there was a notable correlation between the proximity to the nearest food store and the dietary variety of elderly people living in rural areas. These results indicate the necessity of interventions in areas that are at a higher risk of having a limited dietary variety, such as those located far away from food stores. (Gomi *et al.*, 2022). Fushimi *et al.* (2023) has demonstrated that there is a strong correlation between dietary intake of food and nutrients, physical performance, and physical activity among elderly living in urban areas. There was a significant positive correlation between the physical functions (movement function, static balance, and walking function) and the intake of vegetables, seeds, fruits, and milk; as well as with magnesium, potassium and vitamin B6 and with the dietary fiber/carbohydrate composition ratio.

Thus, this article not only provided a compilation of anti-aging foods, but also proposed a suitable dietary pattern for the elderly, taking into consideration any potential restrictions they may have.

4. Anti-Aging Foods for Elderly

Powerful anti-aging foods are enormous sources of essential minerals, vitamins, antioxidants, and bioactive compounds that have a powerful ability to affect the anti-aging process. This article reviews several Asian and Mediterranean foods that affect aging-related diseases.

Curcumin, a polyphenol isolated from natural turmeric, has many pharmacological abilities including antioxidant, anti-inflammatory, anticancer, and neuroprotective activities. Curcumin consumption improves cognitive activity and ameliorates age-related spatial memory deficits among elderly people because the tropomyosin receptor kinase B (TrkB) signals the derived neurotrophic factor upregulation (Farooqui and Farooqui, 2019).

Catechin in green tea (GTC) has a 20-30 times higher level of anti-oxidative activity in comparison to blueberries and strawberries. Recently, GTCs were of high interest in terms of pharmacological activities to prevent age-related neurodegeneration including Parkinson's disease and Alzheimer's disease (Farzaei *et al.*, 2019). Green tea consumption has been wildly suggested to treat age-related diseases in the elderly because of its potential effects on neurodegenerative diseases, markedly Alzheimer's and Parkinson's (Tomata *et al.*, 2016). The main tea polyphenol in green tea is epigallocatechin gallate (EGCG). Its mechanism can disrupt the amyloid plaque formation in the brain parenchyma through multiple pathways, which can be a cause of Alzheimer's disease (Murphy and LeVine, 2010). Green tea consumption for 12 months might prevent an increase of oxidative stress in the elderly but this might not affect cognitive function (Ide *et al.*, 2015).

Garlic’s benefits have a good effect on disorders of the cardiovascular, respiratory, and peripheral nervous systems. The sulfur compounds in garlic act on the signaling pathways of arthritis (Pareek *et al.*, 2019). Also, the bioactive compounds in ginger have therapeutic potential in the treatment of age-related neurological disorders (Choi *et al.*, 2018). Knee osteoarthritis inflammation could be reduced in patients through the supplementation of ginger powder at a dose of one gram per day (Naderi *et al.*, 2015). *Centella Asiatica* was discussed and determined to be the main memory supporter in addition to the research evidence on several mechanisms underlying its neuroprotective properties (Sabaragamuwa *et al.*, 2018).

Perilla seed oil is a rich source of omega-3 polyunsaturated fatty acids, specifically alpha-linolenic acid (ALA) (Dhyani *et al.*, 2019). ALA has a minimal effect on sarcopenia in older men (Cornish and Chilibeck, 2009). Perilla seed oil helps to provide neuroprotection against mild to moderate dementia, although it seems not to improve cognitive function in preclinical studies. Also, perilla seed oil can reduce both total cholesterol and LDL cholesterol. Black sesame seed oil also contains sesamin compounds which are important to the elderly due to their anti-inflammatory and chondroprotective nature. Sesamin has the beneficial effects of lessening apoptosis, astrogliosis, and oxidative stress (Baluchnejadmojarad *et al.*, 2017).

Tropical fruits with anti-aging properties may offer low-calorie *and* rich essential vitamins for the elderly. Avocado includes important micronutrients such as vitamin C, vitamin A, vitamin E, vitamin B6, magnesium, and folate (Weaver, 2013). Furthermore, several phytochemicals were also found in the fruit and seeds of the avocado. Large amounts of alpha-tocopherol and beta-sitosterol were found in avocado pulp oil (dos Santos *et al.*, 2014). Alpha-tocopherol has an effect on the formation of ROS in the inflammatory processes and enhances immune function (Capuron *et al.*, 2009) .Also, beta-sitosterol contributed to suppressing carcinogenesis (Bouic, 2002). The bioactive compounds in avocado could prevent cardiovascular disease and reduce cholesterol (Duarte *et al.*, 2016). Moreover, dragon fruits are also tropical super fruits because of their many health benefits including the ability to improve digestion, fortify the immune system, prevent diabetes, and decrease the risk of breast cancer (Poolsup *et al.*, 2017).

Colored or pigmented rice grains include mainly red, purple, and black rice. They include a variety of antioxidant substances including flavones, tannins, phenolic, sterols, oryzanols, and essential oils (Poomipak *et al.*, 2018). Gamma oryzanol is an especially important antioxidant found in colored rice bran and rice bran oil, which is mainly composed of trans-ferulic acid esters of lipophilic phytosterols (Sing *et al.*, 2015). According to studies, black and purple rice have shown contain two to three times more gamma oryzanol than white rice (Boonsit *et al.*, 2010). Saenjum *et al.* (2012) reported that purple rice bran from glutinous cultivars in Northern Thailand had ability to inhibit nitric oxide production and act as a lipophilic radical scavenger more effectively than a hydrophilic radical scavenger. Suantai *et al.* (2022) demonstrated that red jasmine rice harvested in Chiang Mai, Thailand had antiviral activity against both herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), as well as exhibiting anticancer properties. Thus, it was suggested that purple rice bran is a powerful natural compound with antioxidant, anti-inflammatory and anticancer properties. A large amount of data has indicated the potential for there to be anti-inflammation, anticancer, and anti-aging processes due to the major components found in pigmented rice, especially germ and bran extracts (Limtrakul *et al.*, 2019). Rice berry (*Oryza sativa*), purple-pigmented rice, could significantly prevent memory impairment and reduce the risk of Alzheimer's disease (Pannangrong *et al.*, 2011).

4.1. Natural Antioxidants

The elderly and their caregivers have raised safety concerns about the use of synthetic antioxidants in convenient food products. The uptake of natural antioxidants from foods such as fruits, vegetables, and herbs is the best choice for the elderly because of their safety compared to synthetic antioxidants (Li *et al.*, 2014). Generally, natural antioxidants can scavenge free radicals and maintain the balance of homeostasis in the human body. It is common knowledge that these natural antioxidants assist in the reduction of chronic diseases due to their health-promoting qualities. Lourenço *et al.* (2019) classified natural antioxidants from plants into three main classes, phenolic compounds, vitamins, and carotenoids. Common natural antioxidants from dietary sources may have the potential to be therapeutic in relation to age-related conditions. An example is given in Table 1.

Table 1 Some dietary antioxidants and their functions to delaying in age-related diseases.

Antioxidant Compounds	Natural Sources	Age-related diseases.	References
Carotene	carrots, yellow papaya, spinach, sweet potato and pumpkin	Eye diseases	(Kowluru *et al.*, 2014)
Vitamin C (Ascorbic acid)	citrus fruits, spinach, garlic, cabbage, cantaloupe and strawberries	Cardiovascular disease and cancer	(Passoni and Coelho, 2008)
Vitamin E (α-Tocopherol)	vegetable and seed oils, nut and legumes, avocado	Lung, skin and prostate cancers	(Quin *et al.*, 2005)
Lycopene	red tomatoes, watermelons, papaya, pink grapefruit and pink guava	Alzheimer's disease and Parkinson's disease	(Liu et al., 2013; Min and Min, 2014)
Flavonoids	apple, onions, green tea, red wine, soy products and black beans	Cardiovascular disease, arthritis, and Alzheimer's disease	(Khan *et al.*, 2014; Zhang *et al.*, 2014)
Coenzyme Q10	oily fish (such as salmon and tuna), organ meats (such as liver), and whole grains.	Coronary artery diseases	(Lee *et al.*, 2013; Saini, 2011)

4.2 Sirtuin Activator Foods

Sirtuin foods seem to have become a trendy new diet scheme associated with sirtuins and longevity. The dietary activators of Sirt1 have been widely investigated recently. Polyphenols such as resveratrol and anthocyanin have been reported to activate AMPK, an effect that may activate SIRT (Villalba and Alcain, 2012). Resveratrol, a SIRT1 activator, may be used for the prevention of obesity, tumorigenesis, and aging-related deterioration in heart function and neuronal loss. It has been illustrated that resveratrol can enhance the lifespan through inducing and activating SIRT1 activity and increasing the SIRT1 affinity for both NAD+ and the acetylated substrate. Furthermore, CR with a resveratrol intake exhibited similar anti-aging activities related to SIRT1 and showed an improvement in cognitive performance (Rouse and Egan, 2018) .Piceatannol is a natural source. It is a hydroxylated derivative of resveratrol that has been used as a food additive and herbal medicine in Asia. Additionally, it has been shown to have anti-cancer properties (Potter *et al.*, 2002; Yokozawa and Kim, 2007)

Recent research has indicated that sirtuin-activating compounds such as luteolin, butein, piceatannol, fisetin, quercetin, and resveratrol have a stimulatory effect on the Sir2 activity in yeast. Some of the research reports indicate that a dietary intake of omega-3 fatty acids has the potential to reverse the decline of Sirt1 in rats with a mild traumatic brain injury (Chen *et al.*, 2018). Anthocyanidins can also greatly increase SIRT6 deacetylation activity in vitro. Moreover, cyaniding can upregulate SIRT6 protein expression in human colon adenocarcinoma Caco-2 cells (Rahnasto-Rilla *et al.*, 2018). Interestingly, Khan *et al.* (2021) proposed that SIRT activators can be investigated to determine their effectiveness of alleviating COVID-19 symptoms. Table 2 is a review of the sirtuin-activating compounds that are found in natural food.

Table 2 Sirtuin-activating compounds in sirtuin stimulation and food sources of sirtuin activators.

Sirtuin-activating Compounds	Dietary Sources	References
Resveratrol	grapes, red wine, berries, peanuts, and dark chocolate	(Allard *et al.*, 2009)
Anthocyanidin	red berries including bilberry, raspberry and cranberry	(Rahnasto-Rilla *et al.*, 2018)
Butein	butea, dahlia, coreopsis and searsia	(Semwal *et al.*, 2014)
Piceatannol	red wine, passion fruit, polygonum cuspidatums, rhodomyrtus tomentosa, white tea and peanut	(Kukreja *et al.*, 2013)
Fisetin	strawberry, apple, persimmon, grape, onion, and cucumber	(Khan *et al.*, 2013)
Quercetin	onions, green tea, green pepper, asparagus, red leaf lettuce and tomatoes	(Nishimuro *et al.*, 2015)
Curcumin	turmeric	(Stanić, 2017)
Luteolin	broccoli, pepper, thyme, celery, parsley, rosemary and bird's-eye chilies	(Miean and Mohamed, 2001; Shamsizadeh *et al.*, 2017)

5. Suggested diet pattern for elderly

5.1 Calorie restriction (CR)

CR is defined as a long-term dietary intervention (Anderson *and Weindruch*, 2012) whereby there is a limit in calories and malnutrition is avoided. CR, which can extend the human lifespan by up to five years, has quietly become accepted among leading researchers (Dar *et al.*, 2012). CR intake and the high physical activity levels of an elderly population of Okinawans generated a low Body Mass Index (BMI) relative to the high plasma levels of DHEA (Dehydroepiandrosterone Hormone). This could reduce the fatalities from age-related diseases such as cancer of the prostate, breast, and colon, and coronary heart disease (Willcox *et al.*, 2007). However, for elderly people, there may not be an understanding of the relationship between obesity and its adverse outcome. The relative risk is associated with BMI failure in old age (Stevens *et al.*, 1998). Besides controlling the balance of physical activity with food supplements, an elderly person should try to have a diet that is diverse and nutrient-rich (Tripathi *et al.*, 2016).

Furthermore, CR- mimetics in elderly people should be examined as a powerful alternative for the maintenance of health. CR-mimetics work with the caloric restriction between activated biochemical pathways in mammalian cells through various polyphenols which include resveratrol, catechins, quercetin, and genistein, which is found in plants (Morris, 2010).

5.2 Balanced intake of macro-micronutrients

However, CR for elderly people should also relate to the contributors of macronutrients which include protein, carbohydrates, and fat as total energy. A good amount of knowledge on the determinants of nutritional intake may help in the early prevention of malnutrition. Macronutrient intake for the elderly in the literature is as shown in Table 3.

Table 3 Contributors of macronutrient intakes as total energy for the elderly person

Contributors of Macronutrient intakes (% calories)			References
Protein	**Carbohydrates**	**Fat**	
10-15	55-60	25-30	(Bureau of Food, 2012)
10-35	55-75	20-35	(Ter Borg *et al.*, 2015)
10-35	45-65	20-35	(Services, 2017)
24-26	61-59	15	(Saito *et al.*, 2018)

The presence of the above disorders may increase or decrease because of dietary adjustments and the calories and micronutrients involved. Vitamin A is important for vision, bone improvement, and immune function. Vitamin D with a calcium intake together with physical activity can improve functional capacity and femoral bone density which can decrease the risk of falls among elderly people (Bunout *et al.*, 2006). A calcium balance is also important for older people because it can maintain bone strength and keep bones healthy during older age. However, it should be noted that vitamin K intake is associated with gastrointestinal symptoms and constipation (Shah *et al.*, 2014) .The current dietary reference intake for vitamin E should be raised for the elderly because it has anti-inflammatory and immune function benefits related to the processes and resistance to infection. Total B vitamin supplementation including vitamins B12, B6, B2 and folate is associated with a mild cognitive function impairment among elderly Koreans (Kim *et al.*, 2014). The elderly population often avoids eating meats and other foods that contain zinc to avoid increasing their blood cholesterol levels due to the many associated recommendations. However, this results in a zinc deficiency among the elderly. Elderly people have to restrict their intestinal absorption, so then they can increase their utilization of refined wheat products, which lack in zinc and other fiber-rich foods that contain phytate (Cabrera, 2015)

5.3 Anti-aging food pyramid

The elderly population should be recommended daily servings consisting of macro and micronutrients, bioactive compounds, antioxidants, and other essential compounds for anti-aging which can be found in this article. Notably, the Anti-Aging Diet Pyramid was applied to intake servings and CR for the elderly (Shakti and Dubey, 2015). The Anti-Aging Diet Pyramid is a food pattern made up of dietary habits that will lead to longevity. The sources of the anti-aging diet are described in Fig 2.

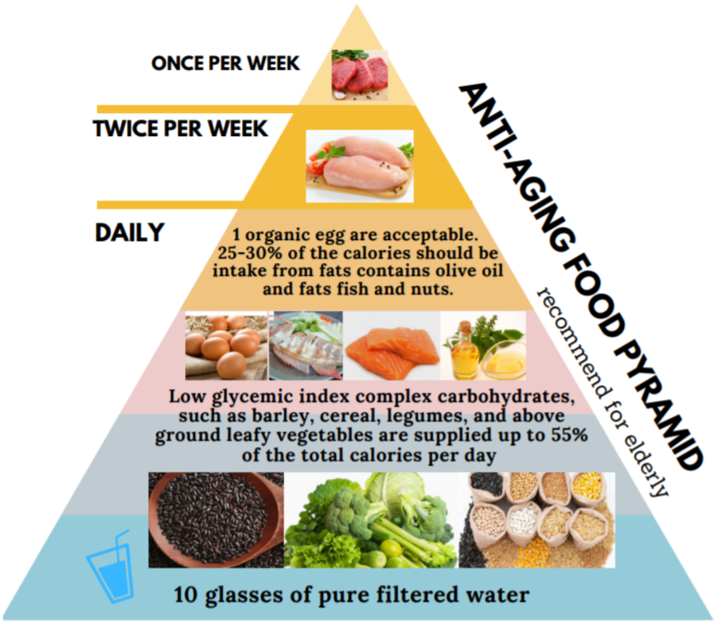

Fig 2 The anti-aging food pyramid can apply to calorie intake for the elderly. Modified from Shakti and Dubey (2015)

The pyramid is divided into 3 layers including daily, once per week, and twice per week. Beginning each day with 10 full glasses of clean and filtered water is the first stage of the pyramid. In addition, complex carbs, which provide up to 55 percentage of the total calories. These carbs are of the low-glycemic index varieties and include barley, grains, legumes, and vegetables with leafy greens. A suitable source of protein for the daily layers of the foundation diet is one egg per day. The majority of the lipids in the diet should come from olive oil, which is rich in monounsaturated fats and an excellent source of antioxidants. According to the anti-aging food pyramid, olive oil, fish, and nut fats should account for 25 to 30 percentage of daily calories. The second layer is a considerably thinner layer that consists of protein-rich foods derived from fish and poultry. The elderly need to eat protein-rich foods from this group at least twice every week. In the third layer, elderly should obtain sweets and lean red meat once a week. This suggested diet pattern can be applied to help develop food products for the elderly to satisfy the balance of micro-macro nutrients that also have a high potential of antioxidants and antiaging ingredients.

The summary of the reviews from multiple research studies linked to diet include calorie restrictions, nutrients, micronutrient intakes, and sirtuin foods in relation to the aging mechanism as shown in Fig 3.

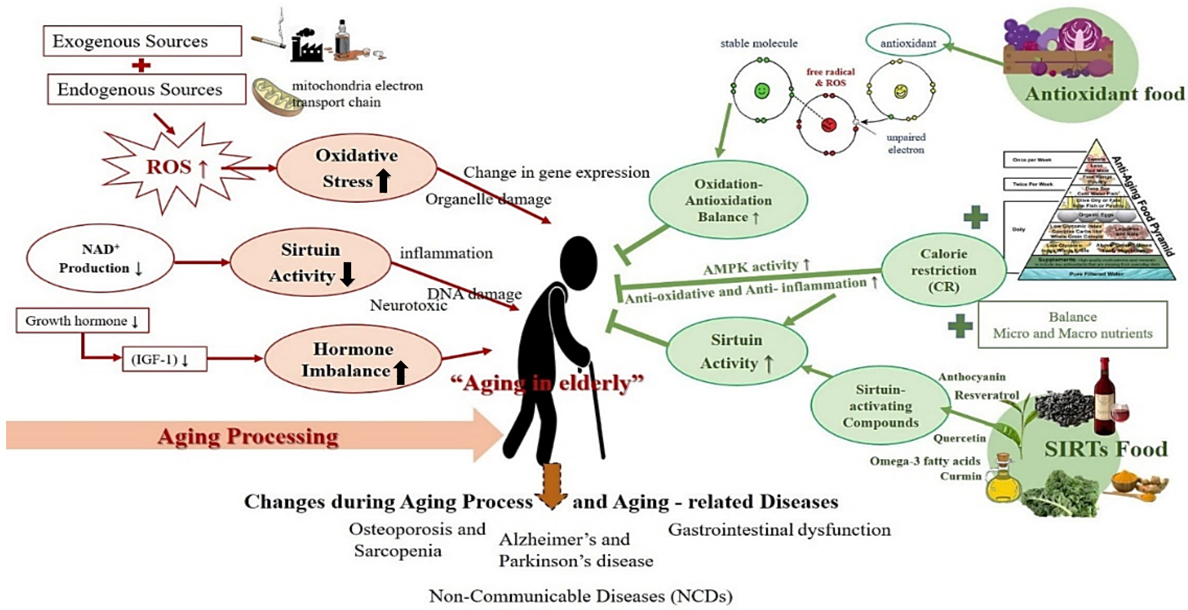

Fig 3 Association between aging mechanisms and benefits of food sources on anti-aging ability.

In red, arrows show the three main aging mechanisms that simulate many disorders in the elderly that consist of increasing oxidative stress, decreasing sirtuin activity, and hormone balance, as described in the previously part. The aging process is linked to increased oxidative stress, ROS can come from both exogenous and endogenous causes. Exogenous or environmental sources such as air pollution, tobacco smoke, ionizing and nonionizing radiation, meals, medications, and xenobiotics (Bhattacharyya *et al.*, 2014). Endogenous sources include cellular metabolic activities such as oxidases (NOXs), nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and xanthine oxidases (XO), among other substances, according to Aranda-Rivera *et al.* (2022). Imai and Guarente (2016) reported that the family of proteins known as "sirtuins" has a special characteristic in that it couples the breakdown of nicotinamide adenine dinucleotide (NAD+) and protein deacylation. This strong relationship between NAD+ and sirtuins has provided invaluable a molecular explanation for how to control aging and longevity in a variety of animals by regulating energy metabolism. According to reports of Poulose and Raju (2015), sirtuin1 activity declines with age, and enhanced sirtuin expression or activation has been linked to longer lifespans. In addition, hormones function as chemical messengers that link and regulate all significant cellular metabolic, growth, and developmental processes. The control of hormonal availability of their active products may be related to the aging process (Monzani *et al.*, 2016). The hormone known as Insulin-like growth factor-1 (IGF-1), which majorly regulates growth hormone (GH)-

stimulated somatic growth, was the subject of numerous investigations. When the mechanism to regulate sex hormones in both men and women ages fails, it may affect musculoskeletal and sexual dysfunction as well as long-term risks of health problems (El-Sakka Ahmed and Hassoba, 2006).

The green text and arrows show the suggested foods and diet patterns that can slow down the aging process and relieve aging symptoms. According to various research, foods with high in antioxidants have an anti-aging effect. It is expected that various antioxidants found in food would help to regulate the redox balance because the body's ability to regulate redox balance is a key aspect of aging (Miyazawa *et al.*, 2022). A recommended diet for the elderly includes caloric restriction along with a balance of micro and macronutrients. CR has the ability to activate AMP-activated protein kinase (AMPK). AMPK is an energy sensor that reduces the kinase activity of the autophagy repressor mechanistic target of rapamycin (mTOR) when CR is present. Moreover, CR both directly and indirectly activates sirtuins, which are nicotine adenine dinucleotide (NAD+)-dependent lysine deacetylases (KDACs) that play essential roles in anti-aging process (Cantó and Auwerx, 2011; Guarente, 2007). According to Kökten *et al.* (2021), CR also induces a change in the composition of the gut microbiota and favors bacteria that produce anti-inflammatory. SIRTs play key roles in the treatment and prevention and age-related metabolic disorders. Various foods contain numerous potential modulators of SIRTs, especially polyphenols, which are anti-inflammatory and anti-aging.

6. Trends when developing food products for the elderly

Developing innovative foods should reach an interesting and increasing segment of the elderly consumer market globally. “Anorexia of aging” refers to their eating and swallowing problems, as well as dry mouth, taste loss, and malnutrition. Many food entrepreneurs need to develop innovative foods for the elderly to meet their nutritional requirements, provide texture-modified foods, and provide acceptable solutions to overcome their eating and swallowing difficulties (Lutz *et al.*, 2019). The nutritive value of the product portions with selected functional ingredients, vitamins, and minerals is the key to developing food products for the elderly. The design product concept of ready-to-eat, easy-to-open, and easy-to-bite and chew foods will support their nutritional and functional needs (Baugreet *et al.*, 2017; Rusu *et al.*, 2020).

Healthy texture modified (TM) or texture-softening foods for the elderly are a major challenge for product development. TM foods refer to foods that have been physically or chemically modified to create a softer texture, which is recommended for dysphagia diets as it reduces the risk of choking. These foods should be soft, moist, elastic, smooth, and easy to swallow (Cichero *et al.*, 2013; Yoshioka *et al.*, 2016). For elderly with dysphagia, replicating human tactile perception in terms of hardness, adhesiveness, and cohesiveness can be utilized for the development of a food products (Nishinari and Fang, 2018). Costa *et al.* (2019) explained the phenomenon of food texture alteration typically involves a step of dilution with water or other liquids during the blending and/or size reduction processes. In addition, several known technologies can produce TM foods including enzymatic treatments, freeze-thawing infusions, high pressure processing, pulsed electric fields, and sonication (Aguilera and Park, 2016). For example, Ma *et al.* (2019) applied treatment sequences of a high pressure processing and papain combination can significantly influence the tenderization effect on yak meat. Chao *et al.* (2022) conducted research to alter the consistency of chicken surimi by combining it with mealworm protein isolate, and coaxial three-dimensional printing was utilized to create a fibrous structure suitable for consumption by the elderly. It is remarkable that this study was able to successfully modify the texture of surimi to create a novel food product with a fibrous shape suitable for elderly consumption.

In addition, numerous studies have been conducted to improve the extraction efficiency of bioactive compounds that support anti-aging via various pathways, such as antioxidants, anti-inflammatory, and anti-cancer, from natural plants and herbs such as turmeric rhizomes, Jasmine rice bran protein hydrolysate, black and golden garlic, black rice grain, Assam green tea, and Lingzhi (Chuensun *et al.*, 2020; Hunsakul *et al.*, 2022; Pakakaew *et al.*, 2022; Salee *et al.*, 2022; Singh, Srichairatanakool, Chewonarin, Brennan, *et al.*, 2022; Singh, Srichairatanakool, Chewonarin, Prommaban, *et al.*, 2022).

Functional foods are ingredients that offer additional health benefits in addition to their nutritional value or may contain supplements or other components designed to improve health (Grochowicz *et al.*, 2021). Functional food is becoming increasingly attractive to both the elderly and their caregivers as a means of promoting a high quality of life. Numerous studies have proposed and advocated for the use of various functional foods to aid the elderly in enhancing their nutritional status and avoiding deficiencies. Arnold *et al.* (2021) has reviewed that the intake of calcium is essential, and chicken eggshell powder has been applied in food products to provide a high daily calcium content. Recently, the market has seen the introduction of various functional products, such as bread enriched with omega-3, which can help to reduce cholesterol levels due to its high content of soluble fiber, and low-fat muesli yoghurt, which can help to lower blood glucose. (Ashwell, 2004; Kalra, 2003). Similarly, Thai researchers have been interested in exploring and creating functional foods for general consumers, particularly for the elderly. For example, Chompoorat and Phimphimol (2019) formulated a functional cupcake for elderly utilizing red kidney bean flour as a main component with a high concentration of protein and fiber, and consuming a single portion of this cupcake could provide 8% of the Daily Recommended Intake of protein. Samappito (2021) developed purple waxy corn milk yogurt prepared from purple waxy corn which is considered to be one of the potential probiotic foods. Moreover, Ngampeerapong *et al.* (2022) investigated methods to reduce sugar and increase fiber content in Thong Ake, a Thai traditional auspicious dessert composed of sugar, flour, coconut milk, and egg yolk, which could potentially be a risk factor for non-communicable diseases. The mixture of stevioside and sorbitol, as well as resistant starch, were taken into consideration. The Thai dessert developed is suitable for those who wish to restrict their carbohydrate consumption, particularly the elderly.

Consequently, the prospective development of functional foods for the elderly should consider several factors that have been illustrated in Figure 4. The developed functional foods for the elderly should have a high anti-oxidant and anti-aging effect based on a balance of micro and macronutrients. Specifically, the improved healthy texture should be examined because it contributes to the positive emotional state of the elderly when consuming functional foods.

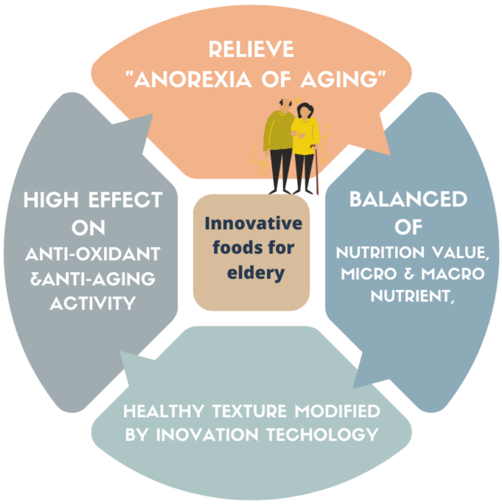

Fig 4 Perspective concept for the functional food development for the elderly.

4. Conclusion

Dietary behavior modification as an anti-aging strategy is essential for the health of the elderly and the prevention of age-related disorders. It is suggested that the diet of the elderly includes daily dietary portions with balanced macro- and micronutrients, bioactive components, antioxidants, and other anti-aging compounds, especially sirtuin activator foods. Due to the variety of anti-aging foods and their benefits, the development of anti-aging products that are considered to be age-related is an interesting topic for the future. The study of the link between innovative anti-aging products and their effects on the aging mechanism, especially in the elderly, should be investigated.

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The efficacy of cinnamon essential oil and vanillin in inhibiting *Colletotrichum* spp. and *Fusarium* spp. isolated from banana fruit

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Abstract

Colletotrichum spp. and *Fusarium* spp. are the main fungi that cause of the postharvest loss of banana fruit loss during storage. In this study, poisoned food bioassay technique and vapor phase diffusion technique were used to investigate the inhibition effect of cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) against *Colletotrichum* spp. and *Fusarium* spp. isolated from infected bananas tissue. For poisoned food bioassay technique, *Colletotrichum* spp. and *Fusarium* spp. were placed on potato dextrose agar containing 20, 100, 200 and 1000 µL/L of C and VC (vanillin 1 g/cinnamon EO 15 mL). The results showed that the growth of *Colletotrichum* spp. and *Fusarium* spp. were delay in PDA containing C and VC at 20 µL/L and above. Both strains were completely inhibited in PDA containing 100 µL/L C and VC and above. For the vapor phase diffusion technique, the 20, 100, 200 and 1000 µL of C and VC were dropped into sterile filter paper and adhered on the plate cover. At the lowest concentration studied, vanillin synergized the inhibition effect of cinnamon EO against both strains. VC had better antifungal effect against *Colletotrichum* spp. and *Fusarium* spp. compared with using C alone. Vanillin can be used to limit the stringent odour of cinnamon EO and helped to lower the amount of cinnamon EO required.

Keywords: *Fusarium* spp., *Colletotrichum* spp., Essential oils, Cinnamon, Vanillin

1. Introduction

In 2020, the export of banana from Asia declined by 12.4% (Crumpler *et al.*, 2021) due to the spread of several fungal diseases that damaged bananas. The symptoms of crown rot and anthracnose, caused mainly by *Colletotrichum musae* and *Fusarium* spp., normally represent after harvested (Triest and Hendrickx, 2016). The postharvest treatments are needed to relieve banana losses. Various synthetic chemicals like fungicides such as chloramines, dichloramines, and trichloromethanes (Fallanaj *et al.*, 2013) have been used in the last few decades to reduce the post-harvest losses (Kumar *et al.*, 2021). However, the use of synthetic fungicides is harmful to consumer health, in which chemical residues are presented in the food (López-Fernández *et al.*, 2016). Therefore, natural preservatives were used as alternatives to replace synthetic chemicals. Plant extracts, such as essential oils (EOs), and had antimicrobial properties, resulted in shelf-life extension of food products (Basavegowda *et al.*, 2021). EOs contain highly bioactive substances that are widely used in food to inhibit bacteria, viruses, and fungi. (Bhavaniramya and Baek, 2019). However, the use of essential oils is limited in intense aroma and taste affecting a negative impact on organoleptic properties (De-Montijo-Prieto *et al.*, 2021). The use of EOs in combination with other food additives might diminish these problems and led to the development of new safe and effective natural antimicrobial agents for food preservation.

Cinnamon EO were reported to suppress the growth of fungi. The main active ingredients were Cinnamaldehyde and trans-cinnamaldehyde, which shown antifungal activity (Wu *et al.*, 2017; Duan *et al.*, 2018) by inhibiting cell wall biosynthesis and destroying the cell membrane of *Fusarium sambucinum*. (Wei *et al.*, 2020). Maqbool *et al*. (2010) investigated the antifungal efficacy of cinnamon EO against infection of *Colletotrichum musae* on bananas stored at 13 ± 1°C and relative humidity 80-90% for 28 days. They reported that the maximum concentration of 0.3% cinnamon EO could be used to extend banana shelf life. In addition, cinnamon EO was applied to reduce mycelial growth and complete spore germination of *Colletotrichum acutatum* in kiwifruit (He *et al.*, 2018*). Colletotrichum acutatum* isolated from mango and strawberry is controlled by cinnamon EO. (Danh *et al.*, 2021; Duduk *et al.*, 2015). Fruit rots of avocado, mango, and papaya caused by *Colletotrichum gleosporioides*, *Fusarium solani*, and *Phytophthora palmivora* can be treated with cinnamon EO (Sarkhosh *et al.*, 2018). Cinnamon EO decreased the growth of *Fusarium oxysporum* in vitro and was found to be the most efficient in reducing conidial germination of *Fusarium oxysporum* causing *Fusarium* wilt in strawberry plants (Park *et al.*, 2017) as cinnamaldehyde in cinnamon EO causes irreversible morphological and ultrastructural changes. (Xing *et al.*, 2014).

Vanillin is used as flavour in many food industries. Vanillin is a phenolic aldehyde with many antimicrobial bioactive qualities that inhibit the growth and development of yeast, mold, and bacteria (Amiri *et al.*, 2021). The shelf life of fruit and vegetable can be extended when applying vanillin, such as apples (Chung *et al.*, 2009), strawberries (Yarahmadi *et al.*, 2014), lettuces (Das *et al.*, 2021), tomatoes (Safari *et al.*, 2020) and mango (Jaimun and Sangsuwan, 2019). Previous research applied a combination of cinnamon EO and vanillin in fresh-cut fruits such as Nectarines dipped in calcium ascorbate (AB) and antimicrobial agents (vanillin or cinnamic acid). After fresh-cut nectarines were packed and stored at 5°C for 8 days. AB+vanillin and AB+cinnamic inhibited microbial counts when compared with control and AB-only samples and did not impart off-flavors. (Muche and Rupasinghe, 2011). The use of vanillin (1 or 2g/L) or cinnamic acid (0.15 and 0.3gL^{-1}) dropped on a filter paper placed inside a container with fresh-cut melon stored for 10 days at 5°C shows melon treated with vanillin (2g/L) and all cinnamon treatments had the highest polyphenol levels, lower respiration rate, and Consumers accepted the flavor of antimicrobial-treated melon. (Silveira *et al.,* 2015). The purpose of this research is to investigate the efficacy of cinnamon EO and vanillin in inhibiting *Colletotrichum* spp. and *Fusarium* spp. in bananas.

2. Materials and Methods

2.1 Analysis of the chemical composition of cinnamon essential oil and cinnamon essential oil mixed vanillin

Cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) were used in this study since there are reports that the combination of EO provide synergistic effect compared with using only one essential oil (Muche and Rupasinghe, 2011). The composition of both formulations were analyzed by GC 7890A and a MSD 5975C (EI mode) mass spectrometer (Agilent Technology, USA). A 0.1 µL volume of sample was injected onto the DB5-MS column (30 m × 0.25 mm ID × film thickness 0.25 µm). The oven temperature was set from 50°C to 280°C. The inlet temperature was 250°C and was detected to be 280°C. Helium carrier gas flowed rate 1 mL/min. For the mass spectrometer was a mass range of 50-550 amu/sec.and ms quadrupole 150°C ms source 230°C. Database of mass spectrometer references W8N08 (John Wiley and Sons, Inc., USA).

2.2 Fungal isolation

Ripe bananas were incubated at 30°C until developed dark brown anthracnose lesions and whitish moulds on banana crown rot. *Colletotrichum* spp. and *Fusarium* spp. were isolated from anthracnose lesions and the infected rots on banana fruits. The isolation of both fungi was performed according to the method proposed by Maqbool *et al*. (2010). Ripe bananas were incubated on moist sterile filter paper at room temperature for 7 days. *Colletotrichum* spp. and *Fusarium* spp. were isolated by tissue transplanting method. Lesion area on banana peel was cut into 5 × 5 mm pieces, soaked in 10% v/v sodium hypochlorite solution for 5 min, washed with sterile distilled water two times, and dried on sterile filter paper. Each tissue piece was placed on a plate containing potato dextrose agar (PDA) medium and incubated at room temperature for 7-10 days at 25 ± 2°C. After 7 days of incubation, the corresponding mycelia were then transferred to a new PDA plate.

2.3 Preparation of antifungal agents

Cinnamon EO from Thai-China Flavours and Fragrances Industry Co., Ltd, Thailand; C (100%) and combined cinnamon EO and vanillin (100%) from KH Roberts Pte., Ltd., Thailand; VC at 20, 100, 200 and 1000 µL were prepared. Vanillin and cinnamon EO mixture (VC) were prepared by adding 1 g of vanillin in 15 mL of cinnamon EO, which is the highest amount of vanillin that can be dissolved in cinnamon oil.

2.4 Inhibition effect of cinnamon essential oil and cinnamon essential oil mixed vanillin against *Colletotrichum* spp. or *Fusarium* spp. by poisoned food bioassay technique

Plant extract, C and VC at 20, 100, 200 and 1000 µL were added in 1 L of potato dextrose agar (PDA, Hi-Media, India) using 0.2% tween 80 as emulsifier (Maqbool *et al*., 2010). The PDA mixture was then autoclaved for 15 min at 121°C and poured into plate to let it solidified. The pure culture of *Colletotrichum* spp. or *Fusarium* spp. was pierced with a 5 mm sterilized cork borer and put on solidified PDA. Four pieces of culture were placed on each plate. PDA with no C or VC served as control. Inoculated plates were incubated at 25°C. The mycelial growth diameter (MGD) expressed in cm (Werghemmi *et al*., 2022), was measured every 2 days and calculate mycelial area using the following equation.

$$MGD\ (cm) = (D1 + D2)/2 \qquad (1)$$

D1 = Mycelial growth horizontal diameter
D2 = Mycelial growth vertical diameter
r = Mycelial growth diameter /2
mycelial area = ∏r2

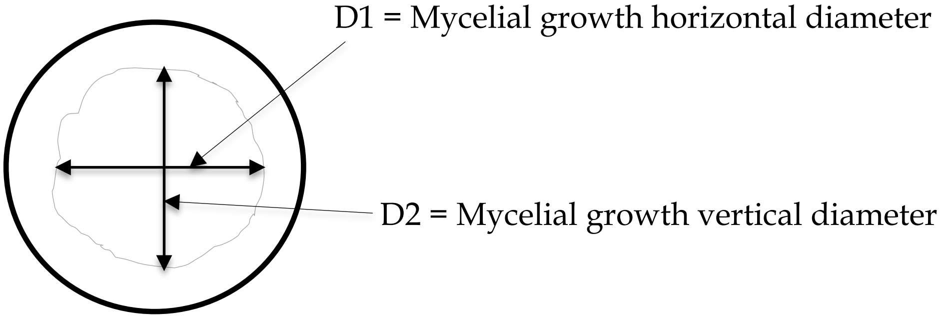

Fig 1 Mycelial growth diameter calculation

2.5 Inhibition effect of cinnamon essential oil and cinnamon essential oil mixed vanillin against *Colletotrichum* spp. or *Fusarium* spp. by vapor phase diffusion technique

The inhibition effect of EOs vaporized in the plate (vapor phase diffusion technique) was used to compare those of poisoned food bioassay (liquid essential oils were mixed in media), since vanillin and cinnamon oil are volatile. Also Sae-Eaw *et al.* (2019) reported that essential oils in vapor phase provided better antimicrobial effect than liquid phase. The fungal activity was tested according to a method proposed by Xing *et al.* (2014) with modifications. Mycelium of *Colletotrichum* spp. or *Fusarium* spp. were pierced with a sterile 5 mm cork borer and four tissue pieces were placed on solidified PDA. Antimicrobial C and VC at 20, 100, 200 and 1000 µL were dropped into a 10 mm in diameter sterilized filter paper disk and placed at the center of a plate. Inoculated plates were incubated at 25°C. Fungal growth was measured every 2 days.

2.6 Statistical analysis

The experiments were conducted with triplicate determinations, and the data were subjected to analysis of variance and Tukey's-b multiple range test ($P<0.05$) using SPSS version 11.0 by SPSS Inc., Chicago, IL, USA software.

3. Results and Discussion

3.1 Chemical Compositions of cinnamon essential oil and cinnamon essential oil mixed with vanillin

Cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) compounds were analyzed using the GC-MS technique. the main chemical compositions of cinnamon EO (C) were 3-allyl-2-methoxy phenol (71.58%) followed by benzoyl benzoate (5.64%), caryophyllene (4.19%), acetyl eugenol (3.91%), trans-cinnamyl acetate (2.23%), β-linalool (2.21%), cinnamaldehyde (1.45%), and safrole (1.41%). While the main chemical compositions of VC were 3-allyl-2-methoxy phenol (66.82%) followed by benzaldehyde,3-hydroxy-4-methoxy (7.41%), benzoyl benzoate (5.22%), caryophyllene (3.91%), δ-cadinene (3.63%), trans-cinnamyl acetate (2.09%), β-linalool (2.00%), cinnamaldehyde (1.33%) and safrole (1.30%). VC contains benzaldehyde, 3-hydroxy-4-methoxy is the main active compound of vanillin. (Sun *et al.,* 2022)

3.2 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on Colletotrichum spp. by poisoned food bioassay technique

Table 1 shows the effectiveness of cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) in inhibiting *Colletotrichum* spp. using the poisoned food bioassay technique. During the first 2 days, no growth of *Colletotrichum* spp. in all treatments was observed. On day 4, the area of *Colletotrichum* spp. on the control plate (with no antifungal agents C and VC) significantly expanded compared with other treatments. The average area of mycelium in control plate was 6.25 ± 1.59 cm^2, while those on the PDA mixed with 20 µL/L C, and VC were only 1.83 ± 2.97 cm^2 and 1.77 ± 0.31 cm^2, respectively. The mycelium areas of *Colletotrichum* spp. treated with 20 µL/L C and VC were statistically significant differences from control on Day 4 to Day 8, indicating that 20 µL/L C and VC suppressed the mycelial growth. In addition, C and VC at concentrations of 100 µL and above completely inhibited *Colletotrichum* spp. However, the effect of C and VC at the same concentration were not significantly different. Many studies reported the effectiveness of cinnamon EO in inhibiting mould. Maqbool *et al.* (2010) found that the 0.4% cinnamon EO in PDA reduced mycelia growth of *Colletotrichum musae,* which was isolated from bananas and inhibited the conidial germination by 83.2% to all other concentrations (0.1, 0.2, 0.3 and 0.4%). Danh *et al.* (2021) reported that cinnamon EO inhibited the development of *Colletotrichum acutatum* isolated from mango. Cinnamon EO 1.6 µL/mL showed the highest antifungal activity compared with basil, lemongrass, orange, mint, and coriander leaves. Sarkhosh *et al.* (2018) also evaluated the effect of cinnamon EO on mycelium growth of *Colletotrichum gloeosporioides* isolated from mango at 25°C and found that cinnamon EO at 1000 µL/L was effective in inhibiting mycelium growth, in which the cinnamon EO concentration was far higher than our study.

Table 1 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Colletotrichum* spp. by poisoned food bioassay technique

Days	Mycelial Area (cm^2)								
	Control	cinnamon oil in PDA (µL/L)				combined cinnamon oil and vanillin in PDA (µL/L)			
		20	100	200	1000	20	100	200	1000
0	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
2	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
4	$6.25\pm1.59^{B,c}$	$1.83\pm2.97^{A,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$1.77\pm0.31^{A,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
6	$8.50\pm1.11^{C,c}$	$4.90\pm0.00^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$4.91\pm0.27^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
8	$12.26\pm0.32^{D,c}$	$7.48\pm0.60^{C,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$7.07\pm1.10^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$

[A] Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).
[a] Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).
Each data point represents the mean ± standard deviations of three replications.

3.3 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on *Colletotrichum* spp. by vapor phase diffusion technique

Table 2 shows the effectiveness of C and VC in inhibiting *Colletotrichum* spp. using the vapor phase diffusion technique, which was intended to evaluate the antifungal activity of volatile EO. The mycelium area in all treatments remained unchanged during the first two days. On Day 4, *Colletotrichum* spp. in the control plate grew fastest, followed by 20 µL C vapor and 20 µL VC vapor. On Day 8, the control mycelium area was 12.26 ± 0.32 cm^2. While the mycelium area in the plate treated with 20 µL C vapor was 8.54 ± 1.28 cm^2, which was significantly larger than those of 20 µL VC (5.55 ± 1.13 cm^2). The addition of vanillin synergized the inhibition effect of cinnamon EO. At higher concentrations (100 µL and above), both C and VC vapor completely inhibited *Colletotrichum* spp. Many studies have been conducted to investigate the use of EO vapor to inhibit fungi. Hong *et al*. (2015) used cinnamon EO vapor to suppress *Colletotrichum gloeosporioides* in peppers by dripping 8 µL of cinnamon EO on filter paper and stored at 25°C for 10 days, which slightly showed growth and 0% conidial germination compared with control. He *et al*. (2018) demonstrated the effectiveness of cinnamon EO vapor against *C. acutatum* in kiwi at 25°C for 7 days. Vapor of cinnamon EO decreased mycelium growth at a concentration of 0.2 mL/mL and completely inhibited spore germination at 0.175 mL/mL. Furthermore, a study of the effects of cinnamon EO on the morphology of *C. acutatum* showed that cinnamon EO affected cell membranes and cytoplasm, including soluble proteins, sugars, and nucleic acids. Cinnamon EO has a high potential to be used as a natural preservative. The addition of vanillin into the cinnamon EO reduced the mycelium growth as shown in Table 2 and the results agreed with Cava-Roda *et al.* (2021). A synergistic study of vanillin and cinnamon bark oil, cinnamon leaf oil, and clove oil inhibited *L. monocytogenes* and *E. coli* O157:H7. The mixture of vanillin and cinnamon EO increases the antimicrobial effect and could be used EO at a lesser amount. Vanillin is widely used as a flavouring agent in the food and beverage industries. (Vijayalakshmi *et al*., 2019) and developed antimicrobial properties against bacteria, fungi, and yeast. (Triana *et al*., 2019). Therefore, the sensory effects of EO must be minimized by incorporating vanillin.

The poisoned food bioassay technique revealed that incorporation of C and VC in PDA had no significant differences antifungal effects against *Colletotrichum* spp. This technique requires Tween 80 as a surfactant to homogenize the EO to the culture medium. As a result, Tween 80 might reduce the antibacterial activity of EO in PDA emulsions (Ma *et al.*, 2016). When evaluation using the vapor phase diffusion technique, VC was more effectively in inhibiting fungal growth than applying C alone. The mycelium growth in plate treated with VC vapor was slower than those treated with C. Vanillin increased antifungal effect in the vapor technique by which vanillin has aldehyde group as the side group of the benzene ring. It functions effectively in protein and fat due to its hydrophobic nature (Li *et al.*, 2021). Vanillin vapor directly deforms the mycelium and acts on the membrane, resulting in ion gradient distribution and respiration inhibition (Fitzgerald *et al.*, 2004). The cinnamon vapor inhibited energy-generating systems. Both compounds have different sites of action in the microbial cell and thus have synergistic antimicrobial effects (Cava-Roda *et al.*, 2021).

Table 2 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Colletotrichum* spp. by vapor phase diffusion technique

Days	Mycelial Area (cm²)								
	Control	cinnamon oil (µL)				combined cinnamon oil and vanillin (µL)			
		20	100	200	1000	20	100	200	1000
0	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
2	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
4	4.63±0.42^{B,d}	1.76±0.00^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	2.03±1.53^{B,c}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
6	8.33±1.09^{C,c}	4.91±0.00^{C,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	4.99±0.82^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
8	12.26±0.32^{D,d}	8.54±1.28^{D,c}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	5.55±1.13^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}

[A] Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).

[a] Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).

Each data point represents the mean ± standard deviations of three replications.

3.4 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on *Fusarium* spp. by poisoned food bioassay technique

Table 3 shows the effectiveness of C and VC in inhibiting *Fusarium* spp. using poisoned food bioassay technique. During the first two days, the mycelium size in all treatments did not change. On day 4, The mycelium areas of the control PDA and 20 µL/L C and VC began to enlarge. On Day 8, The mycelium area of the control was 10.82 ± 0.39 cm² and did not statistically difference from those treated with 20 µL/L VC (10.10 ± 0.7 cm²). However, the area of *Fusarium* spp. on 20 µL/L C was 5.53 ± 3.66 cm², which was statistically different from control and VC. The results showed that C alone inhibited the growth of *Fusarium* spp. better than VC at a concentration of 20 µL/L. Similar to *Colletotrichum* spp., C and VC at 100 µL or more completely inhibit growth of *Fusarium* spp. Many researchers demonstrated that EOs can inhibit *Fusarium* spp. Horváth *et al.* (2013) studied the effect of mint and cinnamon on the growth of *Fusarium* spp. isolated from wheat using the agar dilution method. Cinnamon EO was proven to be a completely effective antifungal agent at 0.01%.

Subsequently, Xing *et al.* (2014) reported that 20 mL/L cinnamaldehyde damages *F.verticillioides* cells by interfering with the enzymatic processes of cell wall production.

Table 3 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Fusarium* spp. by poisoned food bioassay technique

Days	Mycelial Area (cm^2)								
	Control	cinnamon oil in PDA (µL /L)				combined cinnamon oil and vanillin in PDA (µL/L)			
		20	100	200	1000	20	100	200	1000
0	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$
2	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$
4	$1.52 \pm 0.24^{B,b}$	$0.25 \pm 0.25^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$1.83 \pm 0.00^{A,b}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$
6	$4.47 \pm 0.44^{C,c}$	$4.37 \pm 4.15^{B,b}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$4.91 \pm 0.00^{B,c}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$
8	$10.82 \pm 0.39^{D,c}$	$5.53 \pm 3.66^{B,b}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$10.10 \pm 0.70^{C,c}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$	$0.13 \pm 0.00^{A,a}$

[A] Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).
[a] Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).
Each data point represents the mean ± standard deviations of three replications.

3.5 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on *Fusarium* spp. by vapor phase diffusion technique

Table 4 shows the effectiveness of C and VC in inhibiting *Fusarium* spp. using the vapor phase diffusion technique. The mycelium area of *Fusarium* spp. in the control plate started to expand on Day 4. As storage time increased, the mycelium size increased except the plate of *Fusarium* spp. treated with C and VC vapor at 100 µL and above. On Day 8, the average size of control mycelium, mycelium treated with vapor of C and VC 20 µL were 11.05 ± 0.00 cm^2, 10.10 ± 0.70 cm^2 and 5.45 ± 1.10 cm^2, respectively. VC vapor was significantly more effective than C vapor. The result agreed with Xing *et al.* (2014), who used cinnamon EO and cinnamaldehyde to inhibit growth and analysed the morphological alterations of *Fusarium verticillioides*. A fumigation of 40 mL cinnamon EO could prevent the development of *F. verticillioides*. Romero-Cortes *et al.* (2019) also reported using vanillin in PDA to assess the prevention of *Alternaria alternata* from sorghum and barley disease plants and found that the inhibition of *A. alternata* was observed in 480 hours at a dosage of 750 mg/L. Scanning electron microscopy revealed no conidia production and morphologically abnormal body structure. Some researchers reported the synergistic impact of cinnamon EO and vanillin. The use of cinnamon EO vapor with vanillin to inhibit *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus* consistent with Sun *et al.* (2014).

Table 4 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Fusarium* spp. by vapor phase diffusion

Days	Control	Mycelial Area (cm^2)							
		cinnamon oil in PDA (µL)				combined cinnamon oil and vanillin in PDA (µL)			
		20	100	200	1000	20	100	200	1000
0	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
2	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
4	$2.98\pm1.84^{B,c}$	$1.63\pm0.45^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$2.03\pm1.53^{B,c}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
6	$6.07\pm3.17^{C,c}$	$4.91\pm0.00^{C,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$4.99\pm0.82^{C,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
8	$11.05\pm0.00^{D,c}$	$10.10\pm0.70^{D,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$5.45\pm1.10^{D,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$

[A] Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).
[a] Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).
Each data point represents the mean ± standard deviations of three replications.

The poisoned food bioassay technique indicated that C at 20 µL/L was more effective than VC in retarding mycelium growth. While, vapor phase diffusion technique showed that VC provided better antifungal effect. This might be *Fusarium* spp. may use phenolic compounds as carbon sources for the development of fungal growth (Zhou *et al.,* 2012).

Both *Colletotrichum* spp. and *Fusarium* spp. fumigated with VC had significantly slower growth than those treated with C alone. Therefore, vanillin enhanced the inhibitory effects against both strains. Vanillin and cinnamon EO functioned differently. Cinnamon EO contains cinnamaldehyde, which is an addition to the direct way of action, inhibiting fungal growth (Kowalska *et al.,* 2021) and phenolic compounds in vanillin changed the community structures of *Fusarium* spp. (Derito *et al.,* 2009).

4. Conclusion

The use of cinnamon EO and cinnamon EO mixed with vanillin to inhibit the growth of *Colletotrichum* spp. and *Fusarium* spp. isolated from bananas by poisoned food bioassay technique and vapor phase diffusion technique were demonstrated. The growth of *Colletotrichum* spp. and *Fusarium* spp. was inversely related to the concentration of natural volatile EO used. Cinnamon EO and cinnamon EO combined with vanillin at concentrations of 100 µL/L PDA or 100 µL as vapor or above showed complete inhibition of both strains, evaluating by both techniques. Vanillin synergized cinnamon EO in inhibiting the growth of *Colletotrichum* spp. and *Fusarium* spp in vapor phase diffusion assay. Cinnamon EO combined with vanillin has a potential use to fumigate banana to prevent postharvest disease from *Colletotrichum* spp. and *Fusarium* spp.

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