Seminars in Medical Writing and Education. 2025; 4:442
doi: 10.56294/mw2025442

ORIGINAL

Angiogenic Effects of Selected Food Seasonings on Fertilized Duck Embryo via the CAM Assay

Efectos angiogénicos de condimentos alimentarios seleccionados en embriones de pato fertilizados mediante el ensayo CAM

Christine Mae T. Pulot[1], Vanjoreeh A. Madale[2], Shylza C. Tipalan[1], Mylah V. Tabelin[3], Monera A. Salic-Hairulla[2]

[1]Graduate Student, Department of Science and Mathematics Education, College of Education, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines.
[2]Department of Science and Mathematics Education, College of Education, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines.
[3]Department of Biological Science, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines.

Cite as: Mae T. Pulot C, Madale VA, Tipalan SC, V. Tabelin M, Salic-Hairulla MA. Angiogenic Effects of Selected Food Seasonings on Fertilized Duck Embryo via the CAM Assay. Seminars in Medical Writing and Education. 2025; 4:442. https://doi.org/10.56294/mw2025442

Submitted: 13-08-2024 **Revised:** 02-02-2025 **Accepted:** 09-07-2025 **Published:** 10-07-2025

Editor: PhD. Prof. Estela Morales Peralta

Corresponding author: Christine Mae T. Pulot

ABSTRACT

Introduction: angiogenesis is a vital physiological process involved in growth, development, and wound healing, but it may be adversely influenced by environmental and dietary factors. Despite the widespread consumption of food seasonings, the potential effects of their bioactive components—such as monosodium glutamate (MSG)—on angiogenesis remain understudied. To address this gap, this study aimed to investigate the angiogenic effects of selected commercially available food seasonings using the chorioallantoic membrane (CAM) assay in fertilized duck embryos.
Method: twenty fertilized duck eggs were randomly assigned to four groups: control, Product X, Product Y, and Product Z. A 10 % aqueous extract of each seasoning was applied to the CAM of eggs in the respective treatment groups. Blood vessel formation and embryo viability were assessed after incubation.
Results: a significant reduction in blood vessel development was observed in all treatment groups compared to the control (p = 0,00184). Product Z exhibited the strongest antiangiogenic effect (96,15 % inhibition), followed by Product Y (81,65 %) and Product X (65,14 %). Correspondingly, embryo mortality rates increased in treatment groups, correlating with the degree of angiogenesis inhibition.
Conclusions: the findings suggested that certain food seasonings, particularly those containing monosodium glutamate (MSG) and other additives may impair embryonic vascular development and pose potential embryotoxic risks. The CAM assay proved to be a reliable and ethical in vivo model for evaluating the biological impact of dietary compounds. Further studies are recommended to explore dose-dependent responses and histomorphological changes associated with these seasonings.

Keywords: Angiogenic Effects; CAM Assay; Fertilized Duck Embryo; Food Seasonings.

RESUMEN

Introducción: la angiogénesis es un proceso fisiológico vital que interviene en el crecimiento, el desarrollo y la cicatrización de heridas, pero puede verse afectada negativamente por factores ambientales y dietéticos. A pesar del consumo generalizado de condimentos alimentarios, los posibles efectos de sus componentes bioactivos, como el glutamato monosódico (GMS), sobre la angiogénesis siguen siendo poco estudiados.

Para abordar esta deficiencia, este estudio tuvo como objetivo investigar los efectos angiogénicos de condimentos alimentarios seleccionados disponibles comercialmente mediante el ensayo de membrana corioalantoidea (MCA) en embriones de pato fertilizados.
Método: veinte huevos de pato fertilizados se asignaron aleatoriamente a cuatro grupos: control, Producto X, Producto Y y Producto Z. Se aplicó un extracto acuoso al 10 % de cada condimento a la MCA de los huevos de los respectivos grupos de tratamiento. Se evaluó la formación de vasos sanguíneos y la viabilidad embrionaria tras la incubación.
Resultados: se observó una reducción significativa en el desarrollo vascular en todos los grupos de tratamiento en comparación con el grupo control (p = 0,00184). El Producto Z presentó el mayor efecto antiangiogénico (96,15 % de inhibición), seguido del Producto Y (81,65 %) y el Producto X (65,14 %). En consecuencia, las tasas de mortalidad embrionaria aumentaron en los grupos de tratamiento, en correlación con el grado de inhibición de la angiogénesis.
Conclusiones: los hallazgos sugieren que ciertos condimentos alimentarios, en particular los que contienen glutamato monosódico (GMS) y otros aditivos, pueden afectar el desarrollo vascular embrionario y presentar posibles riesgos embriotóxicos. El ensayo CAM demostró ser un modelo in vivo fiable y ético para evaluar el impacto biológico de los compuestos dietéticos. Se recomiendan estudios adicionales para explorar las respuestas dosis-dependientes y los cambios histomorfológicos asociados con estos condimentos.

Palabras clave: Efectos Angiogénicos; Ensayo CAM; Embrión de Pato Fertilizado; Condimentos Alimentarios.

INTRODUCTION

Angiogenesis, the process of new blood vessel formation from pre-existing vessels, is essential for both physiological functions such as growth and tissue repair, and pathological conditions including cancer and chronic diseases.[1,2] This intricate biological process is predominantly regulated by various signaling pathways, notably those involving hypoxia-inducible factors (HIFs), vascular endothelial growth factor (VEGF), and extracellular matrix (ECM) interactions.[3] HIFs play a crucial role in regulating angiogenesis, as they are activated in response to hypoxic conditions and lead to the expression of genes that promote the formation of new blood vessels.[4,5] The role of angiogenesis in cancer is particularly significant, as it allows tumors to secure sufficient oxygen and nutrient supply, thereby supporting their growth and metastasis.[6,7] Moreover, angiogenic mechanisms are vital for tissue repair and regeneration following injury, facilitating the delivery of necessary nutrients, as supported by research highlighting the importance of angiogenesis in wound healing processes.[5,8] Additionally, angiogenesis involves mechanisms such as sprouting, intussusception, and coalescence, which are influenced by cellular dynamics within the ECM.[9,10] Understanding the biological significance of angiogenesis not only elucidates fundamental processes in health and disease but also informs therapeutic strategies aimed at manipulating angiogenic pathways for improved clinical outcomes.[11,6,12]

The chorioallantoic membrane (CAM) assay is widely recognized as a valuable model for studying angiogenesis due to its unique biological properties and experimental advantages.[13,14] The CAM, a highly vascularized structure of the chick embryo, provides an excellent in vivo environment for observing and quantifying angiogenic processes. Its transparency allows for real-time imaging of blood vessel formation, making it an ideal platform for angiogenesis studies related to cancer, wound healing, and drug development.[13,14] Recent studies have demonstrated the utility of the CAM model in evaluating various therapeutic agents, including anti-angiogenic compounds and cancer treatments, showing significant effectiveness in modulating vascular growth and tumor behavior in xenograft models.[15,16,17] The CAM assay facilitates comprehensive investigations due to its ease of access and manipulation, enabling researchers to assess the effects of specific signaling pathways and therapeutic agents on angiogenesis, as indicated by studies that utilized targeted interventions in cancer biology.[18,19,20] Furthermore, the model's low cost and minimal ethical concerns make it a preferred choice for early-stage biomaterial evaluation and drug testing.[21,22] Overall, the CAM assay remains a powerful tool in translational research, advancing our understanding of angiogenesis and aiding the development of novel therapeutic approaches in various pathological contexts.[23-25]

The fertilized duck embryo serves as a viable subject for the CAM assay, providing a unique platform for studying biological processes including angiogenesis and immune responses.[26] The CAM of avian species, particularly ducks, offers a highly vascularized environment that supports the examination of angiogenic factors and therapeutic agents in vivo, with notable advantages such as transparency and ease of manipulation.[26] Recent literature highlights the application of the duck embryo CAM model in testing the efficacy of angiogenesis inhibitors and evaluating biological responses to external agents, underscoring its potential in translational research and drug development.[27] Additionally, specific studies confirm that the CAM assay can effectively represent the immunological responses distinct to duck embryos.[28,18] As an underutilized yet highly relevant alternative to the more common chicken CAM model, the duck embryo enhances the versatility of CAM assays in biomedical research.[26]

Among dietary substances, monosodium glutamate (MSG)—a common food additive and flavor enhancer—has been shown to exert varying effects on angiogenesis. Several studies report that high doses of MSG are associated with increased oxidative stress and inflammation, which can disrupt normal endothelial function and contribute to vascular complications such as hyperlipidemia and atherosclerosis.[29,30] Experimental data also demonstrate that systemic administration of MSG can induce changes in lipid profiles and endothelial responses, potentially leading to pathological angiogenesis.[29,31] Furthermore, MSG exposure has been linked to elevated levels of pro-inflammatory cytokines that may intensify angiogenic activity, contributing to metabolic disorders such as obesity.[29,30] Nevertheless, other studies suggest that moderate MSG intake may not negatively affect vascular health and might even support appetite regulation and metabolic balance.[32] These conflicting findings suggest a dose-dependent relationship, highlighting the need for further research into the comprehensive vascular effects of MSG.[33,34,35] Despite the widespread consumption of MSG and other food seasonings, their impact on angiogenesis remains understudied, especially in the context of developmental biology and experimental vascular models.

This research is therefore grounded in the need to explore the effects of commonly used food seasonings. Understanding their angiogenic potential may yield valuable insights into their broader health implications—particularly because chronic or excessive exposure to pro-angiogenic or anti-angiogenic compounds could contribute to disease progression or impair tissue regeneration. The CAM assay using fertilized duck embryos offers a promising model to assess these effects. This study aims to investigate the effects of selected commercially available food seasonings on the chorioallantoic membrane (CAM) of fertilized duck embryos. Specifically, it seeks to:

1. Evaluate the angiogenic effects of the selected food seasonings on the CAM of fertilized duck embryos.
2. Analyze the percentage growth and percent inhibition of blood vessel formation among the control group and the three treatment groups (Product X, Product Y, and Product Z).
3. Assess and compare the mortality rate of fertilized duck embryos across the control group and the three treatment groups to evaluate potential embryotoxic effects of the food seasonings.
4. Determine whether there are significant differences in blood vessel formation among the control and treatment groups.

Null Hypothesis (H_0) There is no significant difference in the number of blood vessels formed among the control group and the treatment groups (Product X, Product Y, and Product Z).

Alternative Hypothesis (H_1) There is a significant difference in the number of blood vessels formed among the control group and the treatment groups (Product X, Product Y, and Product Z).

METHOD

Type of Investigation and Research Design

This study employed a true experimental design, specifically a completely randomized design (CRD), to examine the angiogenic effects of selected food seasonings using the chorioallantoic membrane (CAM) assay. Fertilized duck embryos were randomly assigned to control and treatment groups to ensure unbiased allocation and replicability.

Timeline and Location

The experiment was conducted over a 7-day period in March 2025 at the Premier Research Institute of Science and Mathematics (PRISM), Mindanao State University–Iligan Institute of Technology (MSU-IIT), Iligan City, Philippines.

Universe, Population, and Selection Process

The universe consisted of fertilized avian eggs. The study population was fertilized duck (*Anas platyrhynchos domesticus*) eggs obtained from Vera Cruz Farm, Buru-un, Iligan City. From an initial collection of 30 eggs, 20 fertilized eggs were selected using candling, a non-invasive method to identify viable embryos at Day 0. Only eggs showing active embryonic development were included to ensure sample homogeneity.

Product Information

Table 1. Product information among treatments

Characteristics	Product X	Product Y	Product Z
Texture	Dry granules, somewhat coarse salt, slightly powder	Fine granules and powdery	Fine granules and powdery
Color	Yellow	Light yellow	Off white, yellowish pale

Net Weight per sachet	8g	8g	4g
Ingredients	Iodized salt, Flavor enhancers (Monosodium glutamate, Ribonucleotide), Sugar, Garlic, Chicken fat, Onion, Spices, Nature-identical flavor, Chicken meat, Egg yolk	Iodized Salt, Flavor Enhancer (Monosodium Glutamate, Disodium Ribonucleotides), Dextrin (Bulking Agent), Sugar, Garlic, Spices (Onion, Black Pepper), Nature-Identical Meat Flavors, Silicon Dioxide (Anticaking Agent), Chicken Fat, Chicken Extract, Artificial Garlic Flavor	Coconut Oil, Soy Sauce (Water, Soybean, Salt, Preservative [Potassium Sorbate (E202)], Maltodextrin, Flavour Enhancers (Monosodium Gluamate E621, Disodium 5'-inosinate E631, Disodium 5'-guanylate E627), Salt, Cane Sugar, Artificial Chicken Flavour, Dehydrated Vegetables (Carrots, Chives), Acid (Citric Acid E330), Spices (Onion, Garlic), Colour (Caramel Colur E150d), Citrus Flavour.
Nutritional Value			
Energy	3 kcal (<2 % RENI)	2 kcal (<2 % RENI)	3 kcal (<2 % RENI)
Fat	0,04g	0g	0,01g
Saturated Fat	0,01g	0g	0,01g
Trans Fat	0mg	0g	0g
Cholesterol	0mg	0g	0,7g
Sodium	502mg	200mg	143mg
Carbohydrates	0,4g	0	0,48g
Dietary Fiber	0g	0	0,3g
Sugar	0,2g	0	0,25g
Protein	0,3 g (<2 % RENI)	0,3 g (<2 % RENI)	0,3 g (<2 % RENI)

Preparation of Treatment Solutions

Commercially available sachets of Product X, Y, and Z were sourced from local retailers. For each treatment, 1000 mg of the seasoning was dissolved in 10 mL of sterile distilled water to create a 10 % (w/v) solution (equivalent to 100,000 ppm). Solutions were stirred, filtered through sterile paper to remove particulates, and stored in clean, labeled containers at room temperature. These were used immediately to ensure consistency and prevent degradation.

CAM Assay Procedure

On Day 0, the selected fertilized duck eggs were cleaned and incubated at 38 °C with 60 % relative humidity in a portable incubator. On Day 5, 0,5 mL of the appropriate treatment solution was aseptically injected into the air sac of each egg using a sterile syringe. The control group received sterile distilled water. Eggshells were resealed with parafilm and incubated for another 48 hours.

On Day 7, a small window was carefully opened at the blunt end of each egg to expose the CAM. Blood vessel formation was assessed visually and documented. Vessel quantification was performed using ImageJ software by counting vessels within a defined field of view.

Experimental Design and Reproducibility

A completely randomized design was implemented with four groups (Control, Product X, Product Y, and Product Z), each consisting of 5 replicates (eggs). This protocol can be replicated using the same concentrations, incubation parameters, injection technique, and vessel quantification method as described.

Variables

- Independent Variable: Type of treatment (Control, Product X, Y, Z)
- Dependent Variables:
  - Blood vessel count (angiogenesis)
  - Embryo mortality
  - Percent inhibition of blood vessel formation
- Controlled Variables: Incubation conditions (temperature, humidity), solution concentration, injection volume, and exposure time.

Data Recording and Statistical Processing

Data on vessel counts were recorded in Microsoft Excel and statistically processed using SPSS v25. Descriptive

statistics (mean ± standard deviation) were calculated. A one-way ANOVA was used to determine significant differences among groups (α = 0,05).

Methodological Definitions

- CAM Assay: An in vivo technique utilizing the vascularized membrane of avian embryos to assess angiogenesis.
- Angiogenesis: Defined as the observable growth of new blood vessels in the CAM within a predefined field.
- Percent Growth / Inhibition: Indicators of angiogenic activity relative to the control group, based on vessel count differentials.

Ethical Considerations

Though fertilized avian embryos are not classified under animal ethics legislation before a certain developmental stage, all handling followed institutional biosafety and ethical guidelines of MSU-IIT. The use of early-stage embryos in this study minimized ethical concerns. Embryos were humanely disposed of after the experiment.

RESULTS

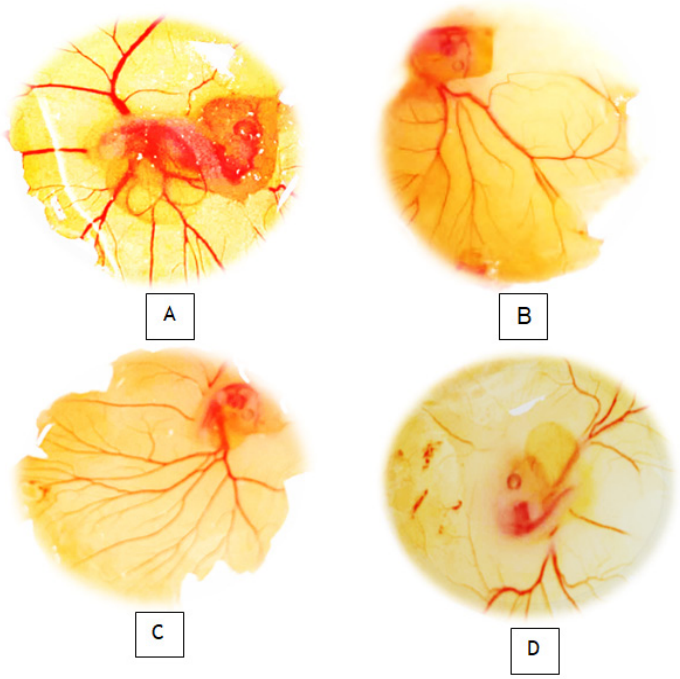

Figure 1. Blood vessel development in duck eggs under different treatments. A. Control – very high branching. B. Product X – slightly reduced branching. C. Product Y – low branching. D. Product Z – very low branching

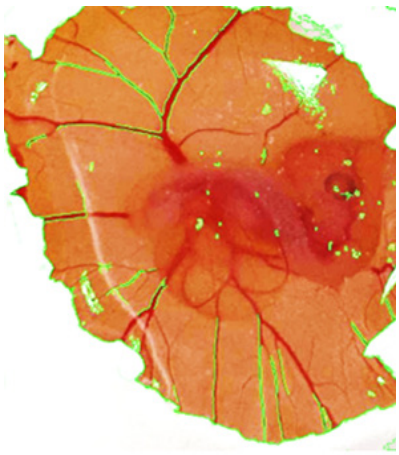

Figure 2. Image shows original color and blood vessel count values annotated using ImageJ multi-point tool

Vessel analysis in angiogenesis entails the quantitative evaluation of blood vessel formation and remodeling within biological tissues. This analytical method is essential for comprehending the dynamics of angiogenesis, a physiological process defined by the development of new blood vessels from existing ones. Figure 2 illustrates the assessment of blood vessel counts 48 hours after the treatment.

Blood Vessel Count

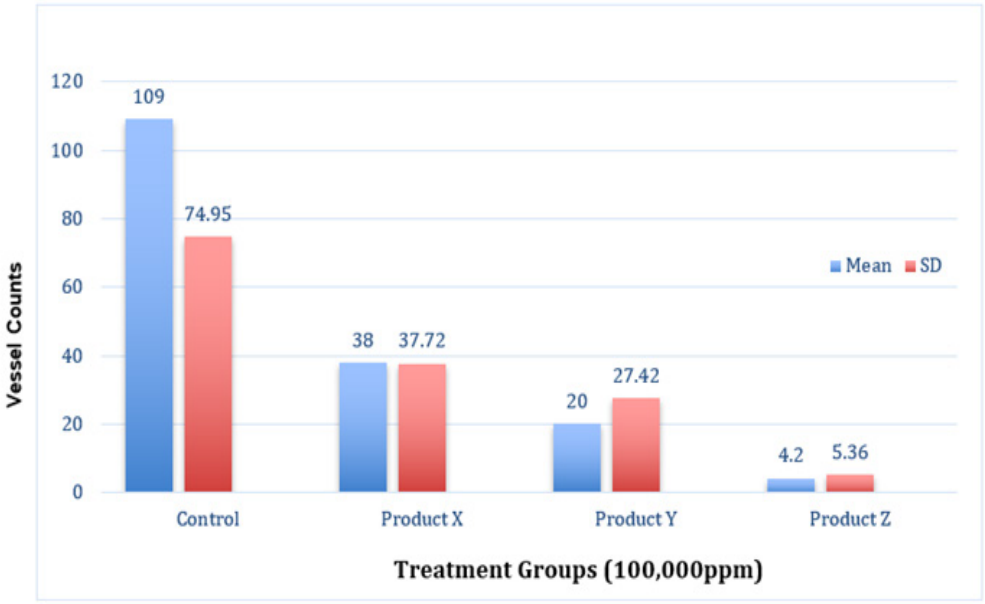

Figure 3. Mean blood vessel count (± SD) among treatment groups at 100,000 ppm concentration

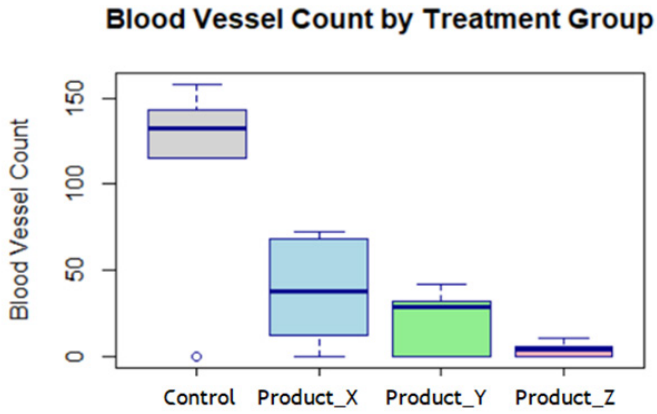

Figure 4. Mean blood vessel count (± SD) for different treatments at 100,000 ppm concentration

Figure 3 displays the vessel counts across different treatment groups, including a control group and three products (X, Y, and Z). Control group has the highest mean vessel count at 109, indicating a robust vascularization compared to the other treatments. Product X group mean vessel count is significantly lower at approximately 74,95, suggesting that this product has some inhibitory effect on blood vessel formation. Product Y group shows an even more reduced mean count of 27,42, indicating a stronger inhibition of vessel formation compared to Product X. With the lowest mean vessel count at 4,2, Product Z appears to have the most substantial inhibitory effect on angiogenesis. Results on the blood vessels counts a clear trend where the control group has the highest vessel counts, while the treatments progressively inhibit blood vessel formation, with Product Z exhibiting the strongest inhibitory effect.

Figure 4 shows box plot of blood vessel count by treatment groups; the Control group promotes the most blood vessel formation. All three products (X, Y, Z) significantly reduced blood vessel counts compared to the Control. Among the products, Product Z showed the greatest inhibition, though its effect is not statistically different from the other two (X and Y). This suggests all three seasonings have a suppressive effect on blood vessel formation, with Product Z showing the strongest (but not uniquely significant) impact.

https://doi.org/10.56294/mw2025442 ISSN: 3008-8127

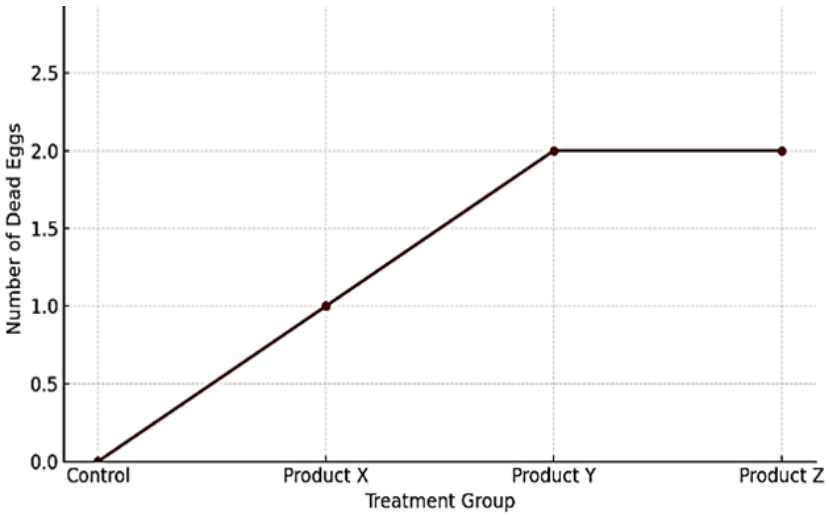

Figure 5. Duck egg mortality in the CAM assay for each treatment group

In a CAM (Chorioallantoic Membrane) assay conducted using duck eggs, embryo mortality was assessed across four treatment groups: Control, Product X, Product Y, and Product Z. The control group exhibited no mortality, indicating that the incubation conditions and handling procedures were not inherently harmful to embryonic development, consistent with established CAM assay protocols. In contrast, one egg died in the Product X group, suggesting a mild adverse effect on embryo viability. Product Y and Product Z both recorded two egg deaths, indicating a more significant impact compared to the control and Product X.

Table 2. Treatment groups and its corresponding percent growth and percent inhibition

Treatment Groups	% growth	% inhibition
Control	100 %	0 %
Product X	-34,86 %	65,14 %
Product Y	-18,35 %	81,65 %
Product Z	-3,85 %	96,15 %

Table 2 outlines the treatment groups along with their corresponding percent growth and percent inhibition values. The control group serves as the baseline, showing a percent growth of 100 % and a percent inhibition of 0 %, indicating normal blood vessel growth without any inhibitory effects. In contrast, Product X exhibits a percent growth of -34,86 %, reflecting a decrease in vessel growth, and a percent inhibition of 65,14 %, suggesting a significant inhibitory effect on angiogenesis. Product Y shows a percent growth of -18,35 %, indicating a reduction in growth that is less severe than that of Product X, while its percent inhibition of 81,65 % demonstrates its effectiveness in suppressing blood vessel formation. Finally, Product Z has a percent growth of -3,85 %, indicating minimal reduction in vessel growth, but it achieves the highest level of inhibition at 96,15 %, suggesting it is the most effective at preventing angiogenesis among the treatments. Results illustrate a trend where each treatment progressively inhibits blood vessel formation, with Product Z being the most effective.

Table 3. One-way ANOVA Summary for Blood Vessel Formation

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Group	3	32,339	10,780	7,93	0,00184	**
Residuals	16	21,796	1,361			
Notes: Significance codes: 0 “0,001 “0,05 ‘,’0,1 “1						

There is a statistically significant difference in blood vessel counts (p = 0,00184) among at least one of the treatment groups (Control, Product X, Y, or Z). This means there is strong evidence to suggest that at least one group differs significantly from the others in terms of blood vessel formation. The p-value of 0,00184 is less than 0,01, indicating a highly significant result. All tested food seasoning products (X, Y, Z) significantly reduced blood vessel formation compared to the control group. However, there is no statistically significant difference among the three products themselves in terms of their inhibitory effect.

DISCUSSION

Food seasonings, particularly certain amino acids and bioactive compounds found in them, can significantly influence angiogenesis during embryonic development. Among these, glutamate has garnered attention due to its critical signaling role in early embryogenesis. Recent research demonstrates that glutamate can activate metabotropic receptors in mouse blastocysts, suggesting a regulatory function in embryo development.[33] This glutamate-mediated signaling is vital for maintaining vascular homeostasis during early growth stages, thereby highlighting its potential influence on angiogenic processes.

Monosodium glutamate (MSG), a widely used flavor enhancer, is one such compound that has been shown to affect embryonic tissues. Notably, MSG can cross the placental barrier, and maternal administration has been associated with adverse histological changes in the placenta, potentially disrupting key angiogenic pathways necessary for normal embryonic development.[34] This underscores the broader importance of dietary components in shaping embryonic vascular outcomes.

In addition to MSG, other agents like lipopolysaccharides (LPS) have also been found to induce angiogenesis in developing embryos. Evidence shows that LPS can trigger vascular proliferation through endothelin receptor interactions, suggesting a complex interplay among nutritional elements, inflammation, and angiogenic signaling during crucial phases of embryogenesis.[35] These findings prompt further investigation into the role of food seasonings and their metabolites in modulating vascular growth during development.

However, it is crucial to recognize that the overall impact of such dietary factors depends on a delicate balance of oxidative stress, cytokine production, and other physiological responses. Studies indicate that fluctuations in these parameters—often triggered by food-based additives—can either enhance or suppress angiogenesis, further illustrating the dual and dose-dependent nature of these effects.[34,29]

This complexity is further evidenced by the results of the current CAM assay, where an increasing trend in embryo mortality was observed from the control group to the treated groups. This suggests that Products X, Y, and Z may exert varying degrees of toxicity or antiangiogenic effects on embryonic development.[37] The higher mortality rates observed in the Product Y and Z groups support the possibility of stronger cytotoxic or angiogenesis-inhibiting properties in these seasonings.[36,38] While Product X appeared less impactful, it nonetheless showed a measurable adverse effect on embryo viability.

These findings point to the need for more detailed analyses to clarify the underlying mechanisms of embryonic mortality. Histological assessments or vascular quantification could help determine whether the observed outcomes result from direct cytotoxicity, angiogenesis disruption, or other forms of developmental interference.[38,37]

The observed antiangiogenic effects align with a growing body of evidence concerning the properties of bioactive compounds in food seasonings. For instance, curcumin from *Curcuma longa* (turmeric) has been shown to downregulate VEGF signaling, impairing endothelial cell proliferation and tube formation.[39,40] Similarly, ß-sitosterol, a phytosterol found in plant-based seasonings, disrupts VEGF pathways and has demonstrated efficacy in reducing pathological neovascularization.[41] Another promising compound, astragaloside IV—derived from traditional nutraceuticals—has been reported to suppress hepatocellular carcinoma-induced angiogenesis by regulating exosome-mediated cellular communication.[42]

Additional plant-derived constituents have also shown antiangiogenic potential. Tylophorine from *Ficus septica*, for example, significantly inhibits VEGF-driven angiogenesis by interfering with endothelial cell proliferation and migration.[43] Likewise, essential oils from *Curcuma phaeocaulis* have demonstrated tumor vessel normalization and reduced aggressiveness through antiangiogenic activity.[44] Together, these findings suggest that compounds present in food seasonings could serve as adjunctive tools in regulating aberrant vascular growth.

Interestingly, the effects of MSG itself remain context-dependent. While some studies highlight its potential to impair angiogenesis via oxidative stress and the upregulation of pro-inflammatory cytokines such as IL-6 and TNF-α, others suggest that MSG may enhance VEGF secretion and aid tissue repair in certain conditions.[45,46] This dual nature illustrates the complex and sometimes contradictory influence of dietary additives on angiogenesis.

In summary, the present findings, supported by the growing body of literature, reinforce the antiangiogenic potential of certain food seasonings and their bioactive components. Incorporating these substances into the diet could provide both preventive and therapeutic benefits in pathological contexts characterized by abnormal blood vessel formation, such as cancer and chronic inflammation. Continued research into these naturally occurring compounds may contribute to the development of novel and accessible angiopreventive strategies.

CONCLUSIONS

This study established that selected food seasonings can modulate angiogenic activity and embryonic viability, indicating their potential influence on vascular development. The findings suggest a formulation-dependent biological response, likely linked to specific additives such as monosodium glutamate. These outcomes affirm

the utility of the CAM assay as a model for preliminary toxicological screening of dietary compounds. The observed effects highlight the relevance of regulating exposure to food additives during sensitive stages of development. Future research should explore concentration gradients and morphological endpoints to deepen understanding of their mechanistic and developmental impacts.

REFERENCES

1. Heer E, Jalving M, Harris A. HIFs, angiogenesis, and metabolism: Elusive enemies in breast cancer. J Clin Invest. 2020;130(10):5074-87. doi:10.1172/jci137552.

2. Veith A, Henderson K, Spencer A, Sligar A, Baker A. Therapeutic strategies for enhancing angiogenesis in wound healing. Adv Drug Deliv Rev. 2019;146:97-125. doi:10.1016/j.addr.2018.09.010.

3. Saman H, Raza S, Uddin S, Rasul K. Inducing angiogenesis, a key step in cancer vascularization, and treatment approaches. Cancers. 2020;12(5):1172. doi:10.3390/cancers12051172.

4. Zhang R, Yao Y, Gao H, Hu X. Mechanisms of angiogenesis in tumour. Front Oncol. 2024;14:1359069. doi:10.3389/fonc.2024.1359069.

5. Lu E, Yang X, Wang T, Huang X, Chen Y, Wang R, et al. Biomimetic thermo-sensitive hydrogel encapsulating hemangiomas stem cell derived extracellular vesicles promotes microcirculation reconstruction in diabetic wounds. Adv Funct Mater. 2023;33(45):2304250. doi:10.1002/adfm.202304250.

6. Kretschmer M, Rüdiger D, Zahler S. Mechanical aspects of angiogenesis. Cancers. 2021;13(19):4987. doi:10.3390/cancers13194987.

7. Korablev A, Sesorova I, Sesorov V, Vavilov P, Mironov A, Zaitseva A, et al. New interpretations for sprouting, intussusception, ansiform, and coalescent types of angiogenesis. Int J Mol Sci. 2024;25(16):8575. doi:10.3390/ijms25168575.

8. Eelen G, Treps L, Li X, Carmeliet P. Basic and therapeutic aspects of angiogenesis updated. Circ Res. 2020;127(2):310-29. doi:10.1161/CIRCRESAHA.120.316851.

9. Chen Q, Xu Q, Zhu H, Wang J, Sun N, Bian H, et al. Salvianolic acid B promotes angiogenesis and inhibits cardiomyocyte apoptosis by regulating autophagy in myocardial ischemia. Chin Med. 2023;18:85. doi:10.1186/s13020-023-00859-w.

10. Chen L, Wang S, Feng Y, Zhang J, Du Y, Zhang J, et al. Utilisation of chick embryo chorioallantoic membrane as a model platform for imaging-navigated biomedical research. Cells. 2021;10(2):463. doi:10.3390/cells10020463.

11. Chu P, Koh A, Antony J, Huang R. Applications of the chick chorioallantoic membrane as an alternative model for cancer studies. Cells Tissues Organs. 2021;211(2):222-37. doi:10.1159/000513039.

12. Saeed S, Hassan A, Suliman A, Moustafa A, Alali F. Methanolic leaves extract of Ziziphus spina-christi inhibits cell proliferation and migration of HER2-positive breast cancer via p38 MAPK signaling pathway. Int J Mol Sci. 2025;26(2):654. doi:10.3390/ijms26020654.

13. Azevedo F, Lopes D, Zóia M, Correia L, Saito N, Fonseca B, et al. A new approach to inhibiting triple-negative breast cancer: In vitro, ex vivo and in vivo antiangiogenic effect of BthTx-II, a PLA2-asp-49 from Bothrops jararacussu venom. Biomolecules. 2022;12(2):258. doi:10.3390/biom12020258.

14. Nitzsche B, Rong W, Goede A, Hoffmann B, Scarpa F, Kuebler W, et al. Coalescent angiogenesis-Evidence for a novel concept of vascular network maturation. Angiogenesis. 2021;25(1):35-45. doi:10.1007/s10456-021-09824-3.

15. Wang J, Chen Y, Wang Q, Xu H, Jiang Q, Wang M, et al. LncRNA SPRY4-IT1 facilitates cell proliferation and angiogenesis of glioma via the miR-101-3p/EZH2/VEGFA signaling axis. Cancer Med. 2022;12(6):7309-26. doi:10.1002/cam4.5517.

16. Chen S, Chen L, Niu Y, Geng N, Feng C. AEG-1 promotes angiogenesis and may be a novel treatment target for tongue squamous cell carcinoma. Oral Dis. 2020;26(5):876-84. doi:10.1111/odi.13300.

17. Miura K, Koyanagi-Aoi M, Maniwa Y, Aoi T. Chorioallantoic membrane assay revealed the role of TIPARP in lung adenocarcinoma-induced angiogenesis. Cancer Cell Int. 2023;23:45. doi:10.1186/s12935-023-02870-5.

18. Aryani R, Nugroho R, Manurung H, Rulimada M, Maytari E, Siahaan A, et al. Anti-angiogenic activity of Ficus deltoidea L. Jack silver nanoparticles using chorioallantoic membrane assay. F1000Res. 2023;12:544. doi:10.12688/f1000research.130477.1.

19. Ribatti D, Annese T, Tamma R. The use of the chick embryo CAM assay in the study of angiogenic activity of biomaterials. Microvasc Res. 2020;131:104026. doi:10.1016/j.mvr.2020.104026.

20. Faihs L, Firouz B, Slezak P, Slezak C, Weißensteiner M, Ebner T, et al. A novel artificial intelligence-based approach for quantitative assessment of angiogenesis in the ex ovo CAM model. Cancers. 2022;14(17):4273. doi:10.3390/cancers14174273.

21. Schneider-Stock R, Ribatti D. The CAM assay as an alternative in vivo model for drug testing. Adv Exp Med Biol. 2020:303-23. doi:10.1007/164_2020_375.

22. Qiu J, Li M, Su C, Liang Y, Ou R, Chen X, et al. FOXS1 promotes tumor progression by upregulating CXCL8 in colorectal cancer. Front Oncol. 2022;12:894043. doi:10.3389/fonc.2022.894043.

23. Kundeková B, Máčajová M, Meta M, Čavarga I, Bilčík B. Chorioallantoic membrane models of various avian species: Differences and applications. Biology. 2021;10(4):301. doi:10.3390/biology10040301.

24. Aventurado C, Billones J, Vasquez R, Castillo A. In ovo and in silico evaluation of the anti-angiogenic potential of syringin. Drug Des Devel Ther. 2020;14:5189-204. doi:10.2147/DDDT.S271952.

25. Cao P, Nie G, Luo J, Hu R, Li G, Hu G, et al. Cadmium and molybdenum co-induce pyroptosis and apoptosis via the PTEN/PI3K/AKT axis in the livers of Shaoxing ducks (Anas platyrhynchos). Food Funct. 2022;13(4):2142-54. doi:10.1039/d1fo02855c.

26. Banerjee A, Mukherjee S, Maji B. Monosodium glutamate causes hepato-cardiac derangement in male rats. Hum Exp Toxicol. 2021;40(12_suppl):S359-69. doi:10.1177/09603271211049550.

27. Al-Otaibi A, Mansour N, Elabd H, Esmail N. Toxicity of monosodium glutamate intake on different tissues induced oxidative stress: A review. J Med Life Sci. 2022;0(0):68-81. doi:10.21608/jmals.2022.264345.

28. Alhamed T, Al-Marzook F, Al-Asady A. The harmful effects of monosodium glutamate on blood parameters, liver and kidney functions in adult white rats and the protective role of omega-3. Indian J Forensic Med Toxicol. 2021;15(3):5245-50. doi:10.37506/ijfmt.v15i3.16266.

29. Sodomora O. Morphometric assessment of the effects of monosodium glutamate on the carotid sinus wall: An experimental study. Rep Morphol. 2023;29(1):39-45. doi:10.31393/morphology-journal-2023-29(1)-06.

30. Mateshuk-Vatseba L, Holovatskyi A, Harapko T, Foros A, Lytvak Y. Changes in the structural organization of lymph nodes during short-term exposure to monosodium glutamate. Rep Morphol. 2022;28(4):34-40. doi:10.31393/morphology-journal-2022-28(4)-05.

31. Ibiyeye R, Sulaimon F, Imam A, Adana M, Okesina A, Ajao M. Phoenix dactylifera and polyphenols ameliorated monosodium glutamate toxicity in the dentate gyrus of Wistar rats. Niger J Physiol Sci. 2023;38(1):73-8. doi:10.54548/njps.v38i1.11.

32. Johnlouis O, Ifeanyi N, Oluchukwu O. Consequence of Tetrapleura tetraptera leaves on pro-oxidants, hepatic functions and histomorphology in monosodium glutamate-intoxicated rats. Res J Med Plant. 2022;16(2):37-48. doi:10.3923/rjmp.2022.37.48.

33. Ružić Z, Kanački Z, Žikić D, Uščebrka G, Mirčeta J. Circulation index as a quantitative indicator of angiogenesis in chorioallantoic membrane of chicken broilers. Contemp Agric. 2018;67(2):164-70. doi:10.2478/

contagri-2018-0023.

34. Ishan G, Ezeuko V. Histological assessment of placental development following maternal administration of monosodium glutamate in Wistar rats. J Appl Sci Environ Manag. 2024;28(6):1639-43. doi:10.4314/jasem.v28i6.1.

35. Wang G, Li P, Zhang S, Zhong S, Chu C, Zeng S, et al. Lipopolysaccharides (LPS) induced angiogenesis during chicken embryogenesis is abolished by combined ETA/ETB receptor blockade. Cell Physiol Biochem. 2018;48(5):2084-90. doi:10.1159/000492547.

36. Nowak-Sliwinska P, Segura T, Iruela-Arispe ML. The chicken chorioallantoic membrane model in biology, medicine and bioengineering. Angiogenesis. 2014;17(4):779-804. doi:10.1007/s10456-014-9440-7.

37. Ribatti D. The chick embryo chorioallantoic membrane (CAM): A multifaceted experimental model. Mech Dev. 2023;175:203-15. doi:10.1016/j.mod.2023.103788.

38. Vargas A, Zeisser-Labouèbe M, Lange N, Gurny R, Delie F. The chick embryo and its chorioallantoic membrane (CAM) for the in vivo evaluation of drug delivery systems. Adv Drug Deliv Rev. 2020;174:89-108. doi:10.1016/j.addr.2021.02.002.

39. Pan D, Gong X, Wang X, Li M. Role of active components of medicinal food in the regulation of angiogenesis. Front Pharmacol. 2021;11:594050. doi:10.3389/fphar.2020.594050.

40. Martínez-Poveda B, Torres-Vargas J, Ocaña M, García-Caballero M, Medina M, Quesada A. The Mediterranean diet, a rich source of angiopreventive compounds in cancer. Nutrients. 2019;11(9):2036. doi:10.3390/nu11092036.

41. Qian K, Zheng X, Wang C, Huang W, Liu X, Xu S, et al. ß-Sitosterol inhibits rheumatoid synovial angiogenesis through suppressing VEGF signaling pathway. Front Pharmacol. 2022;12:816477. doi:10.3389/fphar.2021.816477.

42. Liang M, Wang H, Qi H. Astragaloside IV suppresses the effects of hepatocellular carcinoma cells on proliferation, angiogenesis, and invasion in human umbilical vein endothelial cells by controlling exosomes by inhibiting Rab27a. J Food Biochem. 2023;2023:8812742. doi:10.1155/2023/8812742.

43. Nurhidayati L, Nugroho A, Retnoaji B, Sudarsono S, Fakhrudin N. Antiangiogenesis activity study of awar-awar leaf ethanolic extract (Ficus septica Burm. f.) by chick chorioallantoic membrane method. Indones J Pharm. 2021. doi:10.22146/ijp.607.

44. Feng Y, Deng L, Guo H, Zhao Y, Peng F, Wang G, et al. The anti-colon cancer effects of essential oil of Curcuma phaeocaulis through tumour vessel normalisation. Front Oncol. 2021;11:728464. doi:10.3389/fonc.2021.728464.

45. Fiebel P, Ramachandra S, Holton K. The low glutamate diet reduces blood pressure in veterans with Gulf War illness: A CONSORT randomized clinical trial. Medicine. 2023;102(4):e32726. doi:10.1097/MD.0000000000032726.

46. Lázár A, Adams H, Adger W, Nicholls R. Modelling household well-being and poverty trajectories: An application to coastal Bangladesh. PLoS One. 2020;15(9):e0238621. doi:10.1371/journal.pone.0238621

FINANCING

This research received no external funding. All expenses related to materials, data collection, and analysis were personally covered by the authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORSHIP CONTRIBUTION

Conceptualization: Christine Mae T. Pulot, Vanjoreeh A. Madale.
Data Curation: Shylza C. Tipalan, Mylah V. Tabelin.

Formal analysis: Shylza C. Tipalan, Vanjoreeh A. Madale.
Research: Christine Mae T. Pulot, Shylza C. Tipalan.
Methodology: Christine Mae T. Pulot, Vanjoreeh A. Madale.
Project Management: Christine Mae T. Pulot.
Resources: Mylah V. Tabelin, Monera A. Salic-Hairulla.
Software: Shylza C. Tipalan.
Supervision: Monera A. Salic-Hairulla.
Validation: Vanjoreeh A. Madale, Monera A. Salic-Hairulla.
Display: Shylza C. Tipalan.
Drafting – Original Draft: Christine Mae T. Pulot.
Writing – Proofreading and Editing: Vanjoreeh A. Madale, Monera A. Salic-Hairulla.

https://doi.org/10.56294/mw2025442 ISSN: 3008-8127