Agriculture and Food

AIMS Agriculture and Food, 7(3): 461–480.
DOI: 10.3934/agrfood.2022029
Received: 21 January 2022
Revised: 04 May 2022
Accepted: 25 May 2022
Published: 16 June 2022

http://www.aimspress.com/journal/agriculture

*Research article*

# Comparison of fish and mammalian gelatin film properties: A meta-analysis

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**Abstract:** Edible films (EF) are continuously developed as food packaging alternatives due to their biodegradable properties. EF can be made from polysaccharides, proteins, lipids, and composite components. The use of raw materials certainly affects the properties of EF. Some studies reported that mammalian gelatin films were significantly different from fish gelatin films. However, there have been different results among individual studies. Therefore, the present study would like to obtain a valid conclusion across different studies using a meta-analysis approach. Study selection was performed with the PRISMA guideline. There were six relevant studies and 28 data used for meta-analysis. The statistical analysis was calculated by using Hedges'd. The results show that fish gelatin films had significantly lower ($p < 0.05$) tensile strength, elastic modulus, water vapor permeability, and transparency compared to mammalian gelatin films. Besides, there were two additional factors that are also discussed such as different film fabrication methods and gelatin concentration. Those seasonal factors were conducted by using subgroup analysis and meta-regression, respectively. The results described that the film production method, i.e., casting and compression molding significantly effect ($p < 0.05$) the tensile strength and elongation at break. Slightly different from the method, gelatin concentration was significantly affected ($p < 0.05$) the tensile strength, elongation at break, and water vapor permeability.

**Keywords:** edible film; gelatin; gelatin film; meta-analysis; tensile strength; water vapor permeability

## 1. Introduction

The main principle of food packaging is to maintain the quality of food products from physical, chemical, and biological influences. To date, edible film (EF) is still developed in various kind of packaging. EF is a thin layer made of edible material, which first molded as solid sheets then applied as a wrapping on the food product [1]. EF can be placed on or between food components [2]. The raw materials used for EF are mainly from hydrocolloid (polysaccharides and proteins), lipid, and composite.

Hydrocolloids, i.e., polysaccharides and proteins are the most widely used biopolymers in the manufacture of edible films. Proteins are generally superior to polysaccharides due to their ability to form greater mechanical and barrier films properties [3]. Among protein-based biodegradable films, gelatin films have a high potential to be commercially applied as food packaging films due to their good gas barrier properties [4]. Gelatin films possessed more desirable properties than films made by sodium caseinate, potato starch, and carboxymethyl cellulose [5]. Gelatin is a water-soluble protein in which odorless and has a random configuration of polypeptide chains in an aqueous solution.6 Gelatin is obtained from the partial hydrolysis of a fibrous protein called collagen that mainly found in bones, skins, connective tissues of vertebrate and invertebrate animals [6]. It could be mammalian such as bovine and porcine as a raw materials of gelatin manufacture.

Bovine and porcine gelatin are extensively used all over the world due to their relatively lower price and substantial availability [7]. The total gelatin market worldwide from bovine hides and porcine skins as raw materials reach up to 29% and 42%, respectively [8]. However, there are some concerns about gelatin due to some religious issues such as Muslim and Jewish communities that forbid the consumption of any pork-related products, while Hindus do not consume cow-related products [9]. Moreover, the outbreak of Bovine Spongiform Encephalopathy (BSE), foot and mouth diseases, has increased public health-related concerns [7]. As a result, there has been an increasing studies related to alternative raw materials of gelatin such as fish that can be obtained from fish skins, bones, and scales [10]. The utilization of fish by-products as raw materials for gelatin production could help reduce the waste from fishery processing and increase the economic value of fish by-products [11]. However, the use of fish gelatin as a raw material for edible films in food industry is still limited as compared to bovine or porcine gelatin films. The reason for the under usage of fish gelatin can be due to the presence of fishy-off notes and lower gelling ability of fish gelatin [8].

Past studies described that there were a significantly different properties between mammalian gelatin films and fish gelatin films [12,13]. This could be due to the difference of amino acid composition of each sources used, molecular weight, the ratio of the α- and β-chains of the gelatin molecule [8,12]. However, there have been different results among individual experiments which some studies reported that there is no significant difference between mammalian gelatin films and fish gelatin films in terms of tensile strength and water vapor permeability properties [13,14]. Hence, it is necessary to conduct a meta-analysis study to obtain a valid conclusion from various studies. To date, previous meta-analysis study that exist only focused on the gel strength value of mammalian gelatin and fish gelatin as an ingredient in the food industry [8]. Nevertheless, no study has yet analyzed the comparison of the characteristics between mammalian gelatin films and fish gelatin films. Therefore, the objective of this study was to compare the physical and mechanical properties of mammalian gelatin films against fish gelatin films.

## 2. Materials and methods

The present study was performed through several steps, i.e., formulation of the research question, literature search, study selection, data extraction, and statistical analysis [15–17].

### *2.1. Formulation of the research question*

The formulation of PICO was used for quantitative systematic reviews [18]. Each letter of PICO has a meaning as follow, the P for "population" is the subject that given treatment; I for "intervention" is the independent variable or the treatment; C for "comparison" as a control or comparison; and O for "outcome" which is the dependent variable of a relevant measure as the influence of the intervention given. In this study, each of the PICO approach was described as P: edible films, I: fish gelatin films, C: mammalian gelatin films, and O: characteristics physics and mechanics of edible film. In addition, there were other additional factors that need to be considered because they may affect the results of the analysis. These components were methodological variable (method used for gelatin films fabrication) and moderator variable (gelatin concentration). Those components were analyzed through sub-group analysis and meta-regression.

### *2.2. Literature search*

Literature search was carried out by using a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. This stage were conducted by using various databases such as Science Direct, Google Scholar, Wiley Online Library, Proquest, ACS Publication, Taylor & Francis Online, Pubmed, Nature, Springer, and EBSCO. Some simple keywords used were "gelatin film", "mammalian gelatin film", "fish gelatin film", "tensile strength", and "water vapor permeability". Keywords optimization was also conducted by using boolean operators such as "OR", "AND", and "NOT" functions from PICO-type question. Moreover, advance search (i.e. truncations, synonyms, phrases, singular, plural, active, and passive) were also used. Data was compiled in a reference manager using Mendeley Application.

### *2.3. Study selection*

The selection was done based on the inclusion and exclusion criteria. Inclusion criteria is a list of potential or any related papers that can be used to meta-analysis [19]. In contrast, exclusion criteria mostly are unrelated, duplicated, unavailable full texts, or abstract-only paper [15]. The inclusion criteria used in this study were full-text articles that reported about physical and mechanical characteristics of gelatin films without any food additives such as antioxidant and antimicroba, articles used gliserol as plasticizer, articles used mammalian gelatin films as a control or references, no publication year limit, and published in reputable international peer-reviewed journal. Whereas the exlusion criteria used were gray literature (government reports, theses, and dissertations that have not been published), review articles, and incomplete articles (abstract only).

*2.4. Data extraction*

Data were extracted from each selected study into the Microsoft Excel with the information of the authors' name, year of publication, author regions, journal name, journal index, gelatin sources (experimental and control), film fabrication methods, gelatin concentration, gel strength/bloom value, solvent, type of plasticizer, plasticizer concentration, mean (experimental and control), standard deviation or standard error (experimental and control), and number of replications (experimental and control). SD calculations can be performed using the formula SD = SE √n if the SD value is not mentioned in the article [17,20].

*2.5. Statistical analysis*

In meta-analysis, an effect size is needed to combine various articles into a single scale using a set of metrics [21]. The effect size includes information about the magnitude of the effect that exists from each article which can then be calculated from all studies. It could be used to estimate the overall effect size, confidence interval, and to know how significant the effect size is [19]. In this study, effect size as Hedges' d (Standard Mean Difference/SMD) was used for statistical analysis due to its ability to calculate effect sizes regardless of sample size heterogeneity, unit of measurement, statistical test results, and suitability for estimating the effect of paired treatment [16]. All formulas used were as follows [22]:

$$[S(SD\ pooled) = \sqrt{\frac{(N^E-1)(s^E)^2+(N^C-1)(s^C)^2}{(N^E+N^C-2)}}] \quad (1)$$

$$[J(\text{correction factor}) = 1 - \frac{3}{(4(N^C+N^E-2)-1)}] \quad (2)$$

$$[d(\text{effect size}) = \frac{\bar{X}^E-\bar{X}^C}{S}J] \quad (3)$$

$$[V_d(\text{variance of Hedges' d}) = \frac{(N^C+N^E)}{N^CN^E} + \frac{d^2}{(2(N^C+N^E))}] \quad (4)$$

$$[Sd(\text{standard deviation}) = \sqrt{Vd}] \quad (5)$$

where $\bar{x}$ is the mean value, N is the sample size, and s is the standard deviation. Moreover, E and C respectively means experimental group (fish gelatin films) and control group (mammalian gelatin films). Data analysis was calculated using Microsoft Excel 2016 to calculate s, J, d, Vd, and Sd with the precision of the effect size was described using a 95% confidence interval (CI), i.e. d ± (1.96 x sd). Then continued with Meta-essentials tools with random effects model to get the effect size, forest plot, funnel plot, subgroup analysis, and meta-regression. Meta-essentials tools used due to its free, does not require advanced programming skills, and publication bias analysis can be carried out even using a random effects model [23]. Analysis of variance (ANOVA) with Tukey's test was also conducted to see the influence of moderator variables to the parameters.

## 3. Results

### *3.1. Characteristics of articles*

According to the PRISMA flow chart (Figure 1), the initial searches resulted in 1,734 articles. There were 612 eliminated because it is duplicates articles and its remaining 1.122 articles. After reviewing the title and the abstract, 48 articles were selected. In total, 6 out of the 48 articles were eligible for meta-analysis based on the relevancy and sufficient data. Data extraction was carried out using 6 selected articles with 5 parameters, i.e., tensile strength (28 studies), elongation at break (28 studies), elastic modulus (15 studies), water vapor permeability (28 studies), and transparency (16 studies). The articles used were published since 2007 to 2017 from different countries, i.e. Thailand, India, and USA. The compiled data of each parameter are reported in Table 1.

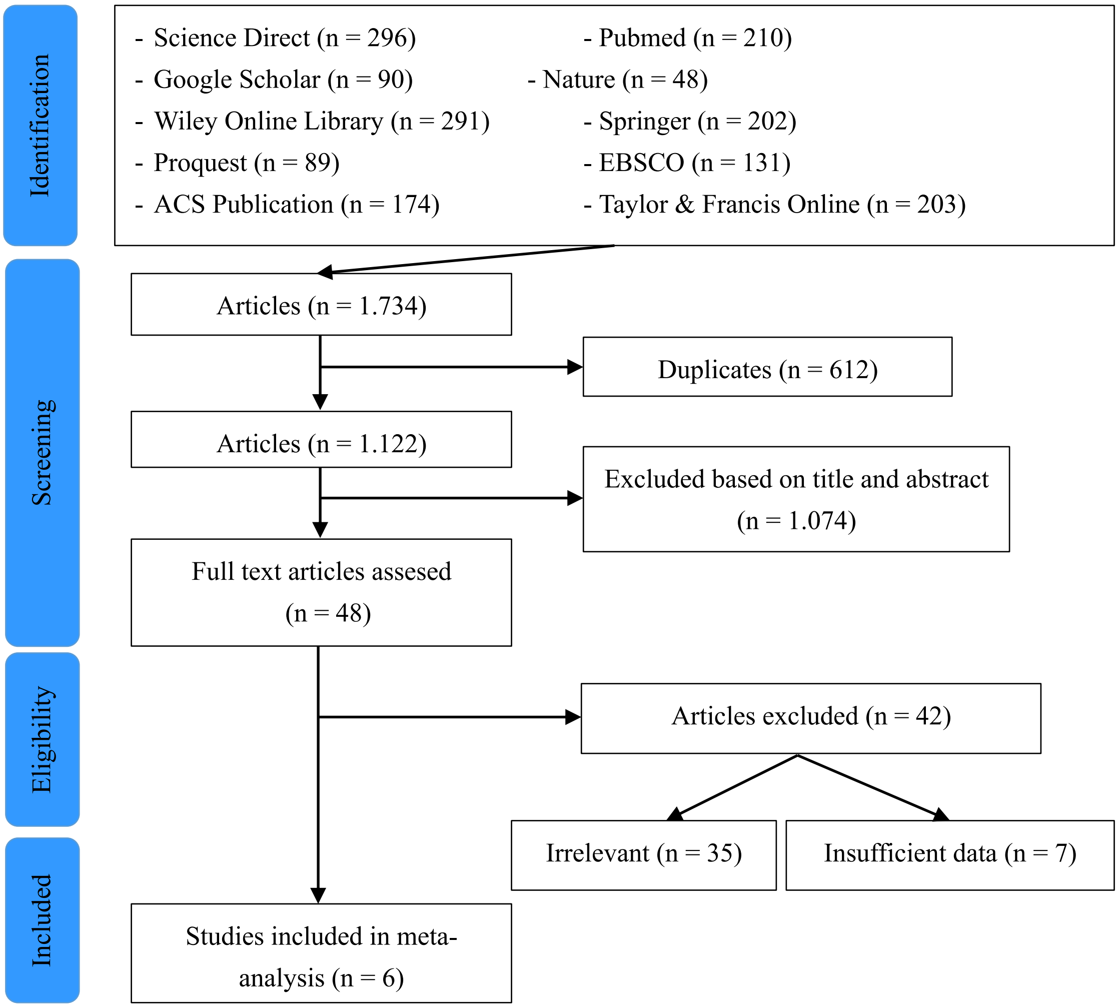


**Figure 1.** The preferred reporting items for systematic reviews and meta-analysis (PRISMA) flow diagram of the literature search process.

**Table 1.** The compiled data used to meta-analysis.

| Study Code | Authors | Experiment Sources | Experiment Mean | Experiment SD[a] | Control Sources | Control Mean | Control SD[a] | N[b] | Film fabrication method | % gelatin (w/v) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. Tensile strength (MPa) | | | | | | | | | | |
| 1 | Chuaynukul *et al*. 2017 | Fish | 25.45 | 1.69 | Bovine | 26.68 | 0.79 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 19.74 | 1 | Bovine | 21.65 | 0.55 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 7.83 | 0.68 | Bovine | 10.24 | 0.85 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 16.67 | 1.63 | Bovine | 20.82 | 2.32 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 13.52 | 0.92 | Bovine | 17.15 | 0.43 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 11.81 | 1.72 | Bovine | 13.85 | 1.33 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 11.42 | 0.56 | Bovine | 11.56 | 1.4 | 3 | Compression molding | 20 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 53.08 | 0.58 | Bovine | 66.11 | 1.94 | 3 | Casting | 6 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 22.41 | 1.67 | Bovine | 24.56 | 0.86 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 14.68 | 0.7 | Bovine | 16.08 | 0.66 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 15.88 | 0.39 | Bovine | 17.5 | 0.4 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 14.88 | 0.55 | Bovine | 16.31 | 0.7 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 17.31 | 0.88 | Bovine | 18.56 | 0.7 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 18.85 | 0.77 | Bovine | 20.1 | 0.69 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 12.62 | 1.24 | Bovine | 14.5 | 0.73 | 3 | Compression molding | 20 |
| 4 | Ninan *et al.* 2010 | Fish | 48.74 | 7.3 | Bovine | 51.68 | 8.6 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 48.74 | 7.3 | Porcine | 63.25 | 6.2 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 48.05 | 4.9 | Bovine | 51.68 | 8.6 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 48.05 | 4.9 | Porcine | 63.25 | 6.2 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 54.92 | 6.7 | Bovine | 51.68 | 8.6 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 54.92 | 6.7 | Porcine | 63.25 | 6.2 | 3 | Casting | 7.14 |
| 5 | Rawdkuen *et al*. 2010 | Fish | 40.74 | 5.18 | Bovine | 32.56 | 6.72 | 5 | Casting | 4 |
| 6 | Zhang *et al.* 2007 | Fish | 48.99 | 4.06 | Porcine | 61.81 | 7.01 | 6 | Casting | 1 |

*Continued on the next page*

| StudyCode | Authors | Experiment | | | Control | | | N[b] | Film fabrication method | % gelatin (w/v) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sources | Mean | SD[a] | Sources | Mean | SD[a] | | | |
| 6 | Zhang *et al.* 2007 | Fish | 8.767 | 0.19 | Porcine | 61.81 | 7.01 | 6 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 62.027 | 5.04 | Porcine | 61.81 | 7.01 | 6 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 18.521 | 3.18 | Porcine | 61.81 | 7.01 | 6 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 10.521 | 6.14 | Porcine | 61.81 | 7.01 | 6 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 19.726 | 6.90 | Porcine | 61.81 | 7.01 | 6 | Casting | 1 |
| 2. Elongation at break (%) | | | | | | | | | | |
| 1 | Chuaynukul *et al*. 2017 | Fish | 4.98 | 0.87 | Bovine | 6.86 | 1.57 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 24.1 | 8.73 | Bovine | 36.29 | 14.67 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 124.21 | 6.79 | Bovine | 125.18 | 11.83 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 31.5 | 5.79 | Bovine | 33.61 | 6.75 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 79.9 | 7.18 | Bovine | 57.87 | 12.96 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 105.52 | 15.1 | Bovine | 126.29 | 11.49 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 82.24 | 12.2 | Bovine | 90.12 | 9.63 | 3 | Compression molding | 20 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 16.93 | 1.22 | Bovine | 25.01 | 2.53 | 3 | Casting | 6 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 26.1 | 1.31 | Bovine | 43.62 | 4.06 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 44.6 | 7.2 | Bovine | 55 | 8.7 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 34.4 | 7.3 | Bovine | 67.2 | 7.4 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 41.6 | 6.88 | Bovine | 70.8 | 5.8 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 33.6 | 5.15 | Bovine | 49.8 | 7.12 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 26 | 5.03 | Bovine | 39.04 | 5.98 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 58.4 | 8.2 | Bovine | 73 | 8.97 | 3 | Compression molding | 20 |
| 4 | Ninan *et al.* 2010 | Fish | 57.16 | 1.4 | Bovine | 30.83 | 0.5 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 57.16 | 1.4 | Porcine | 24.98 | 0.2 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 60.89 | 1.6 | Bovine | 30.83 | 0.5 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 60.89 | 1.6 | Porcine | 24.98 | 0.2 | 3 | Casting | 7.14 |

*Continued on the next page*

| StudyCode | Authors | Experiment | | | Control | | | N[b] | Film fabrication method | % gelatin |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sources | Mean | SD[a] | Sources | Mean | SD[a] | | | (w/v) |
| 4 | Ninan *et al.* 2010 | Fish | 27.00 | 0.7 | Bovine | 30.83 | 0.5 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 27.00 | 0.7 | Porcine | 24.98 | 0.2 | 3 | Casting | 7.14 |
| 5 | Rawdkuen *et al*. 2010 | Fish | 34.14 | 6.07 | Bovine | 24.63 | 10.96 | 5 | Casting | 4 |
| 6 | Zhang *et al.* 2007 | Fish | 21.699 | 0.109 | Porcine | 21.041 | 0.767 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 52.822 | 7.452 | Porcine | 21.041 | 0.767 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 21.479 | 0.439 | Porcine | 21.041 | 0.767 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 21.37 | 0.657 | Porcine | 21.041 | 0.767 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 34.411 | 8.548 | Porcine | 21.041 | 0.767 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 24.11 | 2.52 | Porcine | 21.041 | 0.767 | 3 | Casting | 1 |
| 3. Elastic modulus (MPa) | | | | | | | | | | |
| 1 | Chuaynukul *et al*. 2017 | Fish | 4.38 | 0.24 | Bovine | 5.22 | 0.2 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 3.46 | 0.3 | Bovine | 3.84 | 0.24 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 1.3 | 0.22 | Bovine | 2.05 | 0.16 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 3.63 | 0.07 | Bovine | 6.17 | 1.37 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 2.79 | 0.25 | Bovine | 4.33 | 0.87 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 1.91 | 0.57 | Bovine | 3.48 | 0.71 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 1.6 | 0.36 | Bovine | 2.07 | 0.73 | 3 | Compression molding | 20 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 8.59 | 0.33 | Bovine | 9.58 | 0.39 | 3 | Casting | 6 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 5.19 | 0.51 | Bovine | 5.82 | 0.37 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.67 | 0.14 | Bovine | 2.92 | 0.2 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.92 | 0.13 | Bovine | 3.28 | 0.19 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.82 | 0.16 | Bovine | 2.98 | 0.15 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.94 | 0.13 | Bovine | 3.22 | 0.23 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 3.26 | 0.18 | Bovine | 3.52 | 0.16 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.52 | 0.11 | Bovine | 2.8 | 0.14 | 3 | Compression molding | 20 |

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| Study code | Penulis/tahun | Experiment | | | Control | | | N[b] | Film fabrication method | % gelatin (w/v) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sources | Mean | SD[a] | Sources | Mean | SD[a] | | | |
| 4. Water vapor permeability ($10^{-10}$ g/m s Pa) | | | | | | | | | | |
| 1 | Chuaynukul *et al*. 2017 | Fish | 2.814 | 0.063 | Bovine | 3.239 | 0.047 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 3.372 | 0.032 | Bovine | 3.813 | 0.07 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 3.993 | 0.71 | Bovine | 4.465 | 0.55 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 2.97 | 0.26 | Bovine | 3.29 | 0.26 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 2.61 | 0.08 | Bovine | 3.07 | 0.3 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 2.5 | 0.07 | Bovine | 2.8 | 0.04 | 3 | Compression molding | 20 |
| 1 | Chuaynukul *et al*. 2017 | Fish | 2.25 | 0.06 | Bovine | 2.47 | 0.11 | 3 | Compression molding | 20 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 2.57 | 0.12 | Bovine | 2.81 | 0.14 | 3 | Casting | 6 |
| 2 | Chuaynukul *et al.* 2017 | Fish | 2.84 | 0.07 | Bovine | 3.4 | 0.19 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.74 | 0.18 | Bovine | 3.38 | 0.15 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.86 | 0.18 | Bovine | 3.32 | 0.18 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.96 | 0.11 | Bovine | 3.44 | 0.11 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.76 | 0.18 | Bovine | 3.56 | 0.15 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.7 | 0.19 | Bovine | 3.36 | 0.19 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2015 | Fish | 2.9 | 0.1 | Bovine | 3.35 | 0.15 | 3 | Compression molding | 20 |
| 4 | Ninan *et al.* 2010 | Fish | 2.94 | 0.03 | Bovine | 4.14 | 0.04 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 2.94 | 0.03 | Porcine | 4 | 0.03 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 3.05 | 0.05 | Bovine | 4.14 | 0.04 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 3.05 | 0.05 | Porcine | 4 | 0.03 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 3.67 | 0.02 | Bovine | 4.14 | 0.04 | 3 | Casting | 7.14 |
| 4 | Ninan *et al.* 2010 | Fish | 3.67 | 0.02 | Porcine | 4 | 0.03 | 3 | Casting | 7.14 |
| 5 | Rawdkuen *et al*. 2010 | Fish | 0.91 | 0.06 | Bovine | 0.81 | 0.04 | 5 | Casting | 4 |
| 6 | Zhang *et al.* 2007 | Fish | 3.212 | 0.317 | Porcine | 3.243 | 0.164 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 4.113 | 0.716 | Porcine | 3.243 | 0.164 | 3 | Casting | 1 |

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| Study code | Authors | Experiment | | | Control | | | N[b] | Film fabrication method | % gelatin (w/v) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sources | Mean | SD[a] | Sources | Mean | SD[a] | | | |
| 6 | Zhang *et al.* 2007 | Fish | 3.315 | 0.204 | Porcine | 3.243 | 0.164 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 3.376 | 0.46 | Porcine | 3.243 | 0.164 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 4.092 | 0.01 | Porcine | 3.243 | 0.164 | 3 | Casting | 1 |
| 6 | Zhang *et al.* 2007 | Fish | 5.893 | 1.452 | Porcine | 3.243 | 0.164 | 3 | Casting | 1 |
| 5. Transparency | | | | | | | | | | |
| 1 | Chuaynukul *et al*. 2017 | Fish | 0.65 | 0.03 | Bovine | 0.68 | 0.03 | 3 | Compression molding | 20 |
| 2 | Chuaynukul *et al*. 2017 | Fish | 0.61 | 0.03 | Bovine | 0.64 | 0.019 | 3 | Compression molding | 20 |
| 2 | Chuaynukul *et al*. 2017 | Fish | 0.58 | 0.03 | Bovine | 0.6 | 0.02 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al*. 2017 | Fish | 0.6 | 0.03 | Bovine | 0.64 | 0.03 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al*. 2017 | Fish | 0.63 | 0.02 | Bovine | 0.7 | 0.03 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al*. 2017 | Fish | 0.63 | 0.03 | Bovine | 0.71 | 0.03 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al*. 2017 | Fish | 0.68 | 0.02 | Bovine | 0.81 | 0.02 | 3 | Compression molding | 20 |
| 3 | Chuaynukul *et al.* 2017 | Fish | 0.53 | 0.01 | Bovine | 0.63 | 0.02 | 3 | Casting | 6 |
| 3 | Chuaynukul *et al.* 2017 | Fish | 0.55 | 0.02 | Bovine | 0.65 | 0.02 | 3 | Compression molding | 20 |
| 4 | Chuaynukul *et al.* 2015 | Fish | 0.54 | 0.02 | Bovine | 0.59 | 0.03 | 3 | Compression molding | 20 |
| 4 | Chuaynukul *et al.* 2015 | Fish | 0.55 | 0.03 | Bovine | 0.6 | 0.01 | 3 | Compression molding | 20 |
| 4 | Chuaynukul *et al.* 2015 | Fish | 0.55 | 0.03 | Bovine | 0.59 | 0.02 | 3 | Compression molding | 20 |
| 4 | Chuaynukul *et al.* 2015 | Fish | 0.53 | 0.01 | Bovine | 0.59 | 0.03 | 3 | Compression molding | 20 |
| 4 | Chuaynukul *et al.* 2015 | Fish | 0.54 | 0.01 | Bovine | 0.59 | 0.03 | 3 | Compression molding | 20 |
| 4 | Chuaynukul *et al.* 2015 | Fish | 0.55 | 0.01 | Bovine | 0.61 | 0.01 | 3 | Compression molding | 20 |
| 5 | Rawdkuen *et al.* 2010 | Fish | 3.34 | 0.006 | Bovine | 3.39 | 0.029 | 3 | Casting | 4 |

a)Standard deviation; b)Number of replications.

### 3.2. Primary outcome

#### 3.2.1. Forest plot

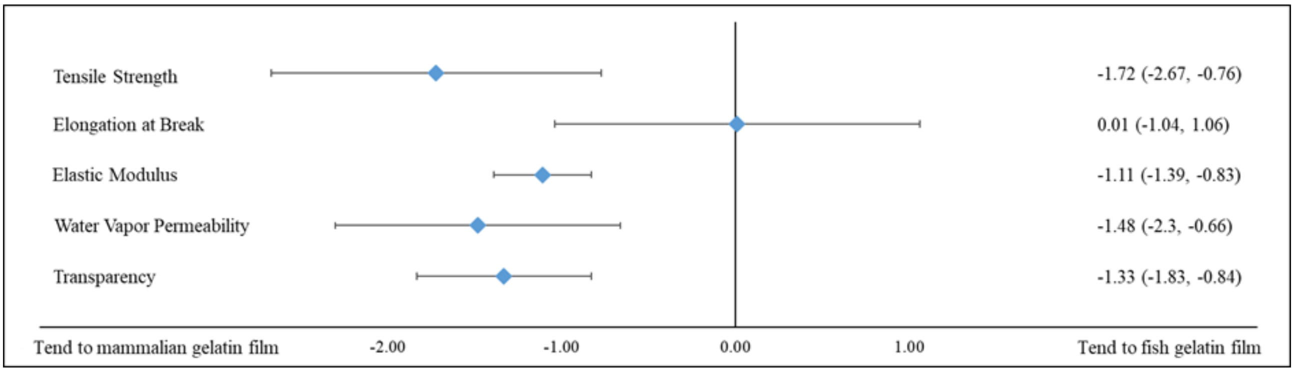


**Figure 2.** Forest plot of cummulative effect size (d++) with 95% confidence interval (CI) of physical and mechanical characteristics between fish and mammalian gelatin films.

According to the forest plot (Figure 2), it can be seen that mammalian gelatin films are significantly different (effect size (95% CI), $I^2$) in terms of tensile strength (−1.72(−2.67 to −0.76), $I^2$ = 72.98%), elastic modulus (−1.11(−1.79 to −0.83), $I^2$ = 0.00%), water vapor permeability (−1.48(−2.3 to −0.66), $I^2$ = 68.14%), and transparency (−1.33(−1.83 to −0.84), $I^2$ = 0.00%) with large effect size, compared to the fish gelatin films. Negative effect sizes indicated that mammalian gelatin film has a higher value of the observed parameters. This could be due to the higher levels of amino acids (proline and hydroxyproline) in mammalian gelatin than fish gelatin.

#### 3.2.2. Funnel plot

Funnel plot, plot of the trials' effect estimates against sample size, was used to assess the publication bias and examine meta-analysis validity [20]. Funnel plots are skewed and asymmetrical in the presence of publication bias and other biases [24]. Moreover, publication bias was calculated using the Begg's test and Egger's test ($p < 0.05$ was considered statistically significant for publication bias) [17].

The result (Figure 3) shows the value of both Begg's and Egger's test were $p < 0.05$ on 4 parameters which consist of tensile strength, elastic modulus, water vapor permeability, and transparency. Furthermore, the value of Begg's and Egger's test on elongation at break characteristic were $p = 0.48$ and $p = 0.01$, respectively.

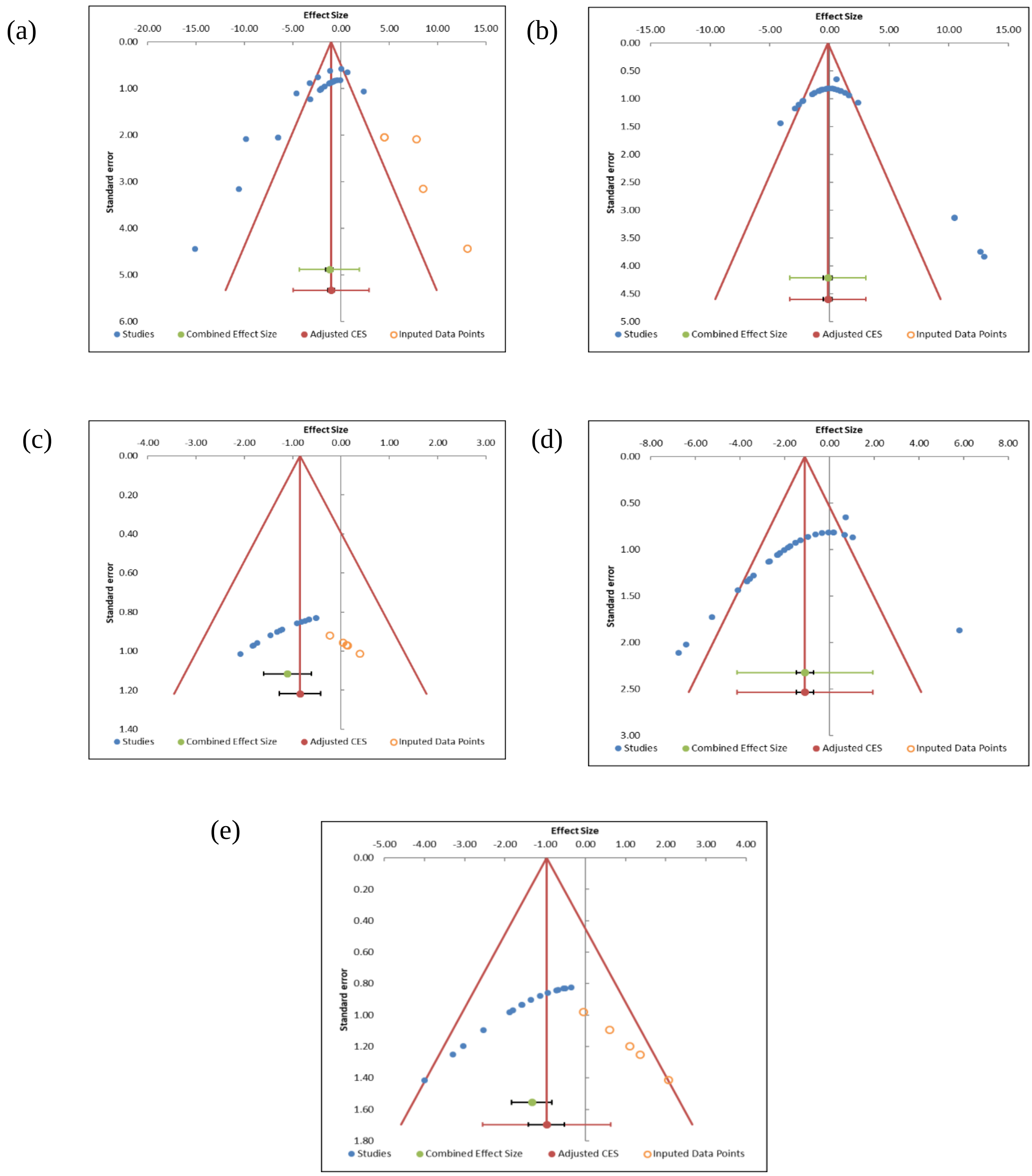


**Figure 3.** The funnel plot from comparison between fish and mammalian gelatin films of 5 parameters (a) tensile strength, (b) elongation at break, (c) elastic modulus, (d) water vapor permeability, and (e) transparency.

### *3.3. Additional factors*

#### 3.3.1. Subgroup analysis

In this study, the variables used for subgroup analysis were the method of edible film manufacture i.e. casting and compression molding (Table 2).

**Table 2.** Effect size for different film fabrication method (sub-group analysis).

| Properties | P-between | d+ (CI 95%) | |
|---|---|---|---|
| | | Casting | Compression Molding |
| Tensile strength | 0.006* | −3.15(−5.42 s.d. −0.87) | −0.97(−1.30 s.d. −0.65) |
| Elongation at break | 0.001* | 1.52(−0.78 s.d. 3.82) | −0.84(−1.45 s.d. −0.22) |
| Elastic modulus | 0.692 | −1.46 | −1.08(−1.38 s.d. −0.78) |
| Water vapor permeability | 0.317 | −1.09(−2.79 s.d. 0.61) | −1.76(−2.33 s.d. −1.18) |
| Transparency | 0.191 | −2.66(−16.28 s.d. 10.96) | −1.22(−1.70 s.d. −0.74) |

*significantly different ($p < 0.05$).

The result showed that different films production method significantly effect ($p < 0.05$) the characteristics of tensile strength and elongation at break. Further analysis was conducted to see the significance effect from the differences film-fabrication methods on tensile strength and elongation properties. The result (Figure 4) show that gelatin films made from casting techniques 226.30% (mammals) and 153.64% (fish) higher tensile strength than gelatin films produced by compression molding method. Otherwise, gelatin films made from compression molding had higher elongation values as much as 154.77% (mammals) and 38.70% (fish) than gelatin films made using casting techniques.

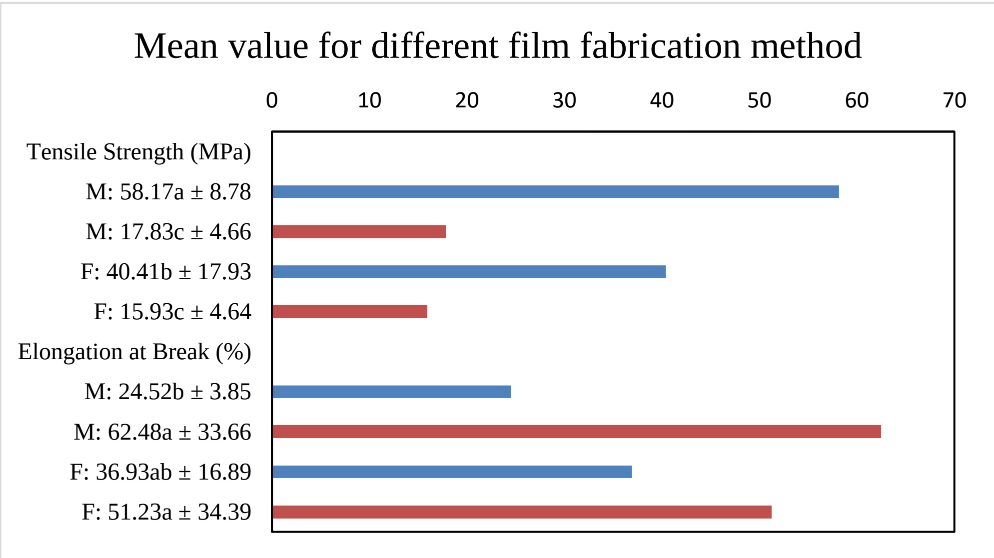


**Figure 4.** Mean value (± standard deviation) of tensile strength and elongation at break for different film fabrication method. Note: M, mammalian gelatin films; F, fish gelatin films; blue bars, casting; orange bars, compression molding; mean values with superscript letters (a–c) are significantly different ($p < 0.05$) according to Tukey's honestly significant difference test.

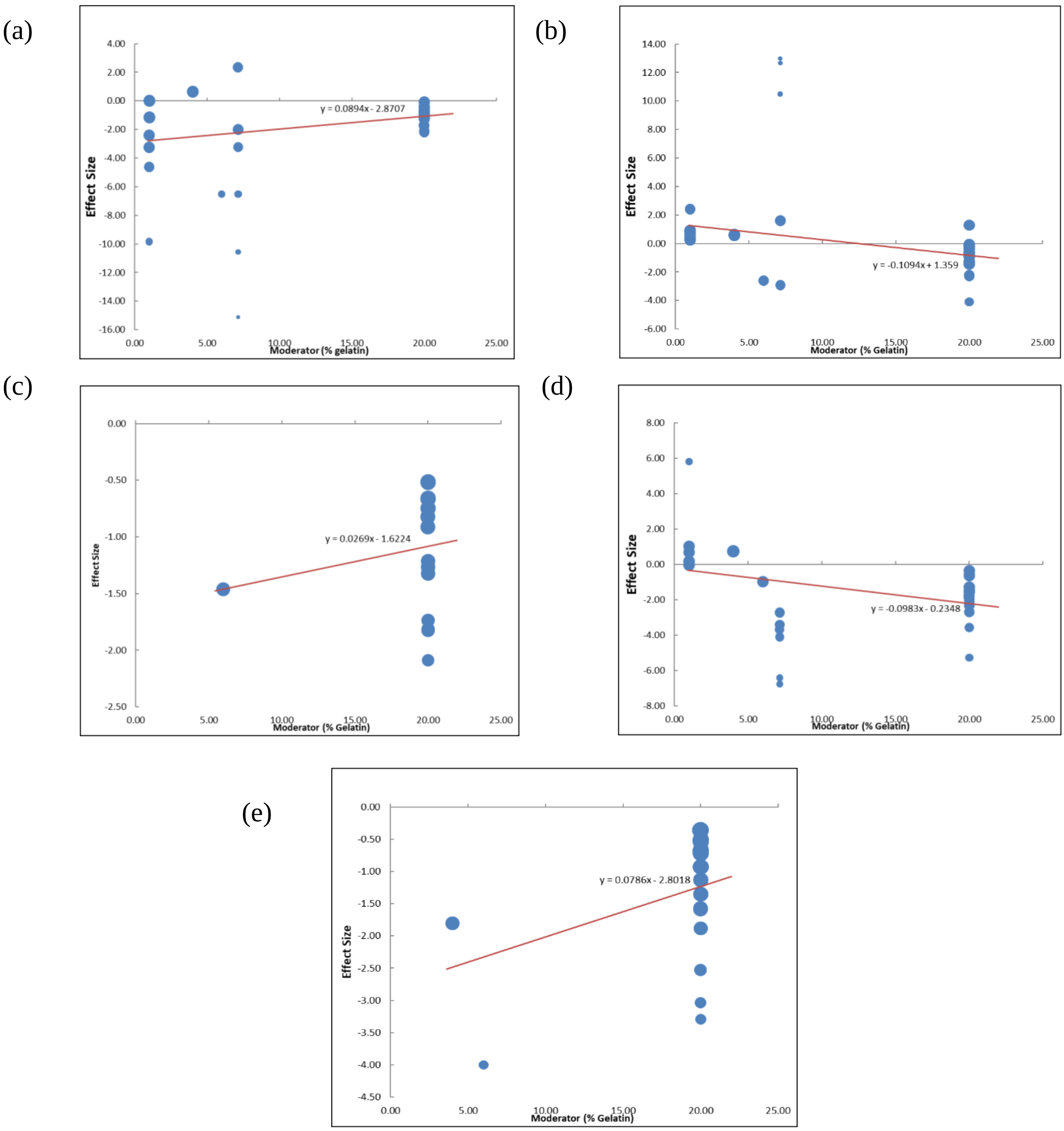


**Figure 5.** Meta-regression effect of gelatin concentration on fish and mammalian gelatin films of (a) tensile strength, (b) elongation at break, (c) elastic modulus, (d) water vapor permeability, and (e) transparency characteristics.

### 3.3.2. Meta-regression

In this study, meta-regression was conducted to describe the effect of gelatin concentration on the effect size of 5 characteristics (Figure 5). Gelatin concentration did not significantly influence the elastic modulus (0.69) and transparency (0.15). However, the concentration of gelatin significantly effect ($p < 0.05$) the characteristics of tensile strength ($p = 0.04$), elongation at break ($p = 0.01$), and water vapor permeability ($p = 0.01$).

## 4. Discussion

### 4.1. Primary outcome

Forest plot convey information about the effect size and confidence interval (CI) across studies. The effect sizes are denoted by the circles or diamonds and confidence intervals by the horizontal lines [19]. CI represents the information about the significance, magnitude, direction of an effect, and is used for inference of an outcome [20]. The calculated effect size is statistically significant ($p < 0.05$) if the range of 95% CI does not reach the zero point on vertical reference line [16,19,24]. The interpretation of effect size was also conducted by looking at the magnitude of the overall effect size using Cohen's benchmarks method. It was used to indicate how large the effect size (d) is. Its effect is divided into 3 classification based on the d value i.e. small (0.2), medium (0.5), and large (0.8) effect size [16]. Furthermore, positive effect size indicates that the parameter observed is greater in the experiment group, and vice versa [16]. The analysis was also carried out by looking at the heterogeneity (I2). It is a percentage of the total variance between studies that range from 0-100%, where 25, 50, and 100 were graded as low, moderate, and high heterogeneity, respectively [25].

This study revealed that mammalian gelatin film has a higher value of tensile strength, elastic modulus, water vapor permeability, and transparency than fish gelatin film due to the amino acid amount. The content of proline and hydroxyproline in carp skin gelatin ranges from 19.16% to 20.86%, meanwhile for bovine and porcine skin gelatin it is 22.91% and 23.7%, respectively [12]. Similar observation were reported that mammalian gelatin contains approximately 30% proline and hydroxyproline, 17-25% for fish gelatin (tilapia, Nile perch, and cod) [25]. During gel formation, proline and hydroxyproline stabilizes the super-helix structure. This stabilization is carried out by control the stearate that established by both the pyrrolidine rings of the amino acids in addition to the hydrogen bonds formed between the amino acid residues [12]. The schematic of the ladder of interstrand hydrogen bonds presented in Figure 6 [26]. Hydroxyproline has an important role in stabilizing the triple helical bonds by forming hydrogen bonds through the OH functional group [27]. The higher the gelatin gel strength, the higher tensile strength gelatin film will be [28]. Besides amino acid composition, the characteristics of edible films can also be affected due to molecular weight distribution, degree of renaturation, and triple helix structure [29,30]. An increase in triple helix levels lead the Young's modulus higher but lower the degree of swelling [31]. In accordance with previous studies which reported that mechanical properties of gelatin films was correlated with the amount of triple-helical content [32].

Film characteristic such as transparency also affected by its thickness. The thicker the film, the higher the transparency value because more light was absorbed on film shot by a spectrophotometer with a certain wavelength [33]. The transparency value indicates the clarity of the film. The high value of transparency indicates edible blurring or decreased clarity [34].

Mammalian gelatin films had a higher mechanical and physical properties compared to fish gelatin films. Higher permeability is detrimental to food quality, it should be a strong consideration for film formulation and processing [13]. According to this result, fish gelatin film might be suitable as packaging for confectionery food products such as candy in which requires higher water vapor barrier capabilities. For this reason, fish gelatin film present better performance to avoid moisture loss. Thus, it is potensial for cheese packaging application due to one of the problem in some types of cheese is the high moisture loss. It might increase cheese hardness and lead to undesirable organoleptic properties [35].

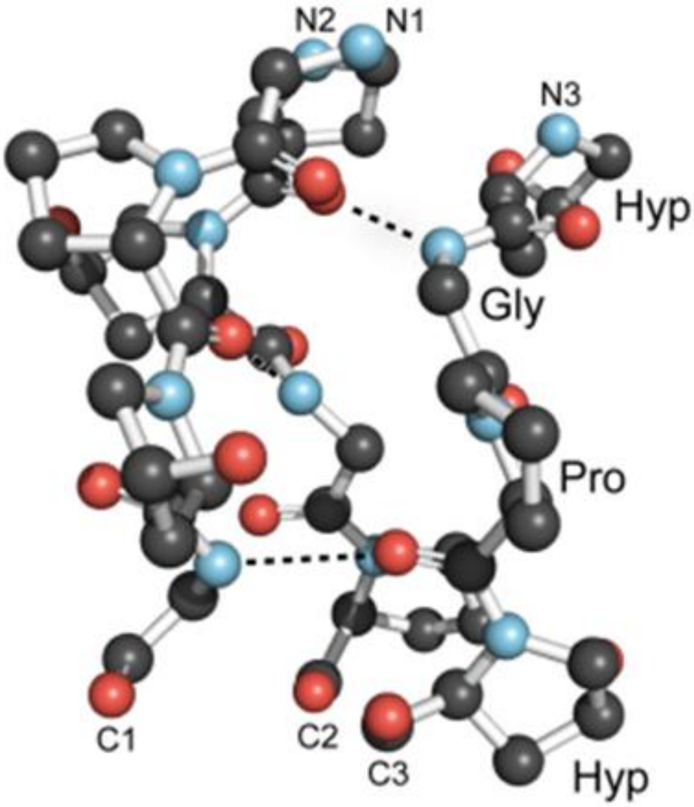


**Figure 6.** The schematic of the ladder of interstrand hydrogen bonds.

*4.2. Additional factors*

Two additional factors were analyzed with subgroup analysis and meta-regression. Subgroup analysis was performed to described heterogeneity and explore differences in effects by partitioning studies into characteristic groups defined by study-level categorical covariates [20]. The subgroup analysis result of this study was in accordance with previous study that revealed gelatin films made by casting process had higher tensile strength characteristics, but had lower elongation values [36]. ELB values exhibited the opposite trend to the TS, the films with the highest TS had the lowest ELB and vice versa [12]. Another study reported that edible films obtained by casting method were stronger and stiffer [37]. This could be due to different molecular characteristics that affect the interaction of gelatin chains in the film matrix. In thermo-compression molding method, the protein structure changes due to denaturation during the heating process. As a consequence, the edible film produced has a shorter peptide chain more than the film processed by the casting technique [38]. Film fabrication with high temperatures process lead to protein degradation, thereby reducing the strength of the film [39]. Molecular arrangement is another factor that influence the formation of 3-dimensional film networks. For films made by casting method, gelatin molecules could undergo partial renaturation in which they could retain their triple helical structures and they have enough time to arrange themselves in such a way that higher intermolecular interactions and a more uniform network were formed [14].

Furthermore, meta-regression was used to describe heterogeneity by looking at the relationship between estimated effect size (vertical axis) and continuous covariates (horizontal axis) [20,40]. The interpretation was determined by the statistical result in which it would be significant if $p < 0.05$.

Moreover, the analysis was also considered by looking at a slope, which a positive slope indicates that effect size and variables positively related, and vice versa. The meta-regression result in this study was in accordance with previous research that reported as gelatin concentration increased, so too did the significant differences between mammalian gelatin films and fish gelatin films in 3 characteristics i.e., tensile strength (from 4% to 8% gelatin concentration), elongation at break strength (at an 8% gelatin concentration), and water vapor permeability strength (at gelatin concentrations of 4% and 6%) [29]. Gelatin films using high gelatin concentrations had good mechanical properties, but lower barrier properties [29].

## 5. Conclusions

The result of meta-analysis illustrated that there was a significant difference in both physical and mechanical properties between mammalian and fish gelatin films. Mammalian gelatin films had a better mechanical propertie compared to fish gelatin films. In contrast, fish gelatin films had a better water vapor permeability compared to mammalian gelatin films. For this reason, fish gelatin films may be suitable for packaging of confectionery product such as candy or cheese. However, it needs to be considered that film production method and gelatin concentration will affect the gelatin films properties such as tensile strength, elongation at break, and water vapor permeability. In order to develop this study, further identical meta-analysis is needed to evaluate the properties of edible film from different fish species (warm-water fish and cold-water fish) gelatin and parts of animals (bones, scales, skins) gelatin.

## Conflict of interest

All authors declare no conflict of interest.

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