

**UNIVERSITI TEKNOLOGI MARA**

**PHYSICOCHEMICAL AND  
FUNCTIONAL PROPERTIES OF  
SOYBEAN TEMPEH PROTEIN  
HYDROLYSATE AND ITS  
APPLICATION IN MEATBALL**

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of the requirements for the degree of  
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## AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

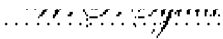
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## ABSTRACT

*Tempeh*, a type of food derived from fermented soybean, has attracted the attention of researchers due to its superior nutritive qualities and metabolic regulatory functions. However, freshly prepared tempeh is easily perishable and can be kept for only 1 – 2 days at 27°C. Overripe tempeh is generally discarded as waste. Tempeh protein, in its original form, has low functionality due to its limited solubility in water. Therefore, to increase the solubility of proteins and alter its functional properties, enzymatic hydrolysis of tempeh to tempeh protein hydrolysate (PH) was suggested. This study was conducted to produce protein hydrolysate from overripe tempeh as functional food ingredients. This study consisted of five phases, i.e., in the first phase, the preliminary analyses on tempeh stored for five days at 27°C comprised of total phenolic content, total flavonoid content, amino acid content, mineral content, nutritional properties, and antioxidant activities (DPPH radical scavenging, ferric reducing antioxidant power,  $\beta$ -carotene bleaching, and oxygen radical absorbance capacity assays). The second phase of the study involved an optimum enzymatic hydrolysis condition of PH by using flavourzyme enzyme, hydrolysis time and enzyme/substrate as independent variables and total flavonoid content (TFC) and glutamic acid content (GAC) as its responses. The techno-functional properties (solubility, foaming, emulsification, and gelation) of PH were determined in the third phase. The fourth phase of the study was conducted to observe the physicochemical property changes of PH and its by-products (tempeh flour, defatted tempeh flour, and tempeh protein isolate) using FESEM, FTIR spectroscopy, DSC, thermo-gravimetric analysis, a spectrophotometric method, and RP-HPLC. The last phase of the study included the determination of the effect of PH as the functional ingredient and also on the storage stability of traditional meatball throughout the nine months storage at -18°C. During this phase, two formulations of meatball were compared, i.e., the meatball formulation with egg (F1) and the meatball formulation with PH (F2). Results of the first phase revealed that further fermentation to 72 h increased the antioxidant activity of tempeh. Therefore, tempeh that undergoes 72 h of fermentation (overripe stage) was selected based on the maximum antioxidant activity showed by the assays. Based on the optimisation study, the optimum condition for the enzymatic hydrolysis of 6.0 g of soy protein isolate from soybean tempeh was at 55°C with 2.6% enzyme to substrate concentration heated for 128 minutes which resulted in 89.3 mg QE/g in TFC and 12.96 g/100g of GAC. The findings for the techno-functional properties showed that the degree of hydrolysis was 20.60%, with 96.42% solubility, emulsification activity index of 65.30 m<sup>2</sup>/g at pH 12, and 70.63% foaming capacity. Moreover, the hydrolysate showed high emulsification stability (70.40%) and foaming stability (49.70%) after 60 min. Results showed that ferulic acid, epicatechin, and daidzin were the major bioactive compounds detected in PH. The substitution of egg by PH in the meatball formulation resulted in significant improvement ( $p < 0.05$ ) in the water holding capacity (WHC), cooking yield, cooking loss, colour, and texture of the meatball. The presence of PH in the meatball (F2) also extended its shelf life up to nine months of frozen storage. The sensory scores by untrained panellists showed no significant difference ( $p > 0.05$ ) between the two meatball for the overall acceptability attributes. Hence, it can be concluded that PH has the potential to be used as a functional ingredient in the meatball.

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