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ENZYMATICALLY HYDROLYSED EGGSHELL MEMBRANES BIOPEPTIDES-BIOCALCIUM COMPLEX CHARACTERISATION, BINDING CAPACITY AND BIOAVAILABILITY

NUR FARAH SYAHIRAH BINTI ABD KADIR

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ABSTRACT

Eggshell membranes (ESM) are considered food waste in the egg processing industry. Recently, researchers have shown an increased interest in enzymatically treated ESM hydrolysate (ESMH). This is because eggshell biopeptides may help to improve eggshell biocalcium bioavailability, and calcium-binding capacity, but the data were limited. Thus, in this study, the ESM, and its hydrolysates (Alcalase-treated eggshell membrane hydrolysate (Al-ESMH), and Trypsin-treated eggshell membrane hydrolysate (Tr-ESMH)) were characterised. Subsequently, the characterisation of the ESM, and ESMHs was carried out using ultra-violet visible spectroscopy (UV-Vis), and Fourier transform infrared spectroscopy (FTIR). The calcium-binding capacity was performed by its protocol. Thereafter, the calcium bioavailability was carried out using *in vitro* equilibrium dialysis through a semipermeable membrane. The calcium quantitation was performed using atomic absorption spectroscopy (AAS). The obtained results from UV spectra showed two intense absorption peaks at 238 nm, and 280 nm. The second peak at 280 nm was used to quantify the soluble protein in ESMHs. The results showed that the Al-ESMH has a higher protein concentration (17.27±0.11 mg/mL) than Tr-ESMH (3.28±0.01 mg/mL), and ESM (2.11±0.19 mg/mL). All three samples showed significant differences of $p < 0.05$. The FTIR spectra of ESMHs suggest the presence of peptides for both treated ESM samples after enzymatic hydrolysis. These can be seen from the C-O skeletal vibration found at 1080.17 cm^{-1}, and C-N stretching around 1240.74 cm^{-1}, and 1236.78 cm^{-1} for both ESMH samples. The results of the calcium-binding capacity studies indicated that the stable pH of pH 8, mass above 10 mg, and 30 min duration of treatment enhanced the calcium-binding of the ESMHs. Al-ESMH demonstrated a statistically increase in calcium-binding capacity than Tr-ESMH. Further, *in vitro* gastrointestinal investigation demonstrated the presence of ESM, and ESMHs increased the Ca bio-absorption of calcium chloride solution (~4 times higher), and ES powder (~15 times higher). Both results were significant at $p < 0.05$ which ES recorded statistically higher Ca bioavailability than $CaCl_2$. Taken together, the finding of the current study suggests that the alcalase, and trypsin hydrolysed the ESM, and changed its chemical structure characteristics. Even though the ESMHs have a low calcium-binding capacity, their role in enhancing calcium bioavailability is recognised.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Over the past century, there has been a dramatic increase in research regarding the importance of the eggshell as one of the natural calcium supplements in the form of calcium carbonate ($CaCO_3$) (Amalraj & Pius, 2015; Bartter et al., 2018; Harvey et al., 1988; Yoo et al., 2009). Calcium carbonate ($CaCO_3$) is usually obtained from limestone; it is also one of the cheapest and most commonly available sources for calcium supplement productions. Even though the limestone- $CaCO_3$ is a common source for nutritional supplementation, but various research reported its bioavailability is poor (Harvey et al., 1988; Nicar & Pak, 1985; Soares Jr, 1995).

Previous research works have established that the eggshell biocalcium has better bioavailability than the calcium processed from the limestone. Studies in rats and piglets have confirmed that the bioavailability of eggshell biocalcium (71.3%) is better than pure calcium carbonate (66.9%) (Hirasawa et al., 2001; Schaafsma & Beelen, 1999). However, limited information is available to elucidate the factors that enhanced the bioavailability of eggshell biocalcium. On the other hand, there are a number of studies have begun to examine the use of eggshell waste as a biological sources for calcium and it may improve the eggshell biocalcium bioavailability more than the calcium from non-biological sources (Al Omari et al., 2016; Hanzlik et al., 2005; Hirasawa et al., 2001; Meiron et al., 2011; Szeleszczuk et al., 2015; Waheed et al., 2019).

The egg is an essential and versatile ingredient that has been used from the old century to the present time in cooking and food processing. It is used in many food productions including baked goods, mayonnaise, salad dressings, fast foods and egg noodles. In between the eggshell and the egg white, there are eggshell membranes (ESM). The ESM consists of a thin inner ESM and a thick outer ESM. The ESM resides between the inner surface of the eggshell and albumen (egg white). The outer ESM consists of mucin protein which allowed for gas transmission (Baláž, 2014; Tsai et al.,