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In-vitro-digestion of a controlled release material: composite aerated gel containing egg white protein

Marnick SRUN[1], Pimnibha HIRUNSORN[1], Kwanruedee WACHIRATTANAPONGMETEE[1], Theparit PITIRIT[1], Supawan THAWORNCHINSOMBUT[1]*

Abstract

This study aimed to produce a healthy jelly-like product containing protein and bioactive ingredient. Egg white protein (EWP: 0, 3, 6% w/w) and sucrose (0 and 7.5% w/w) were mixed at fixed levels of konjac glucomannan, κ-carrageenan, and sodium bicarbonate in an aerated gel preparation. An interaction between EWP and sucrose significantly yielded lower gas hold-up capacity exhibiting less and small pores confirmed by SEM images. The 3% EWP with 7.5% sucrose gel was selected to further study *in vitro* digestion by incorporating rice bran hydrolysate (RBH) and determining phenolic compound bioaccessibility. Syneresis and gas hold-up capacity of the gel was improved due to aeration and RBH ($P<0.05$). Aeration also enhanced the bioaccessibility of phenolic compounds and antioxidants after digestion. Therefore, this study introduces a new food matrix which is an aerated gel containing egg white protein that exhibits high bioaccessibility following pancreatic digestion.

Keywords: Composited aerated gel; egg white protein; microstructure; rice bran hydrolysate; *in vitro* digestion.

Practical Application: Aeration of egg white protein can be an innovative method to create a new food matrix with a controlled release fate for nutrients *in vitro* digestion.

1. Introduction

The increasing number of aged people and decreasing birth rate have led to a growing rate of an ageing society. Thailand is one of the quickest ageing countries in the world, with 11.5% of the total population aged 65 years old and older in 2019 (United Nations, 2019). Aged people can typically face food consumption complications due to anatomical and physiological changes built during aging. Finding suitable food forms for aged people that are safe and nutritious is a challenge. Food with soft and moist textures that require minimal chewing and are easy to swallow, such as gel, is one of the appropriate food forms for aged people (Cichero, 2016).

Recently, there has been great attention on incorporating bioactive compounds into food systems to provide extra-nutritional constituents that give plentiful positive health benefits for consumers. Aerated gels or air bubbles incorporated into the gel system have been considered as one of the delivery systems. Aerated structures may aid in mastication, increase enzyme access to substrates, and improve taste delivery (Zúñiga & Aguilera, 2008). A few research studies employed aerated gels as food matrices to deliver different nutrients. For instance, Tomczyńska-Mleko et al. (2014) studied the controlled release of minerals under simulated gastric conditions. The same group also further explored the release of different concentrations of calcium ions from egg white aerated gels and compared the release between aerated and non-aerated gels in simulated digestion (Tomczyńska-Mleko et al., 2016).

Egg white proteins have a well-balanced amino acid composition which provides abundant health benefits, including supporting muscle growth and nervous systems (Koshinaka et al., 2021). The major component is ovalbumin, followed by ovotransferrin, ovomucoid, and lysozyme (Liu et al., 2017). Owing to its high quality and easily digestible proteins, it can be considered as a good choice of food composition for older people. In addition to its health benefits, egg white is well known for its gelation ability. It can create heat-induced gels to enhance food consistency as well as acting as a medium to deliver nutrients, flavor, and provide a unique texture (Su et al., 2015).

Heating is often necessary to ensure microbiological safety and proper gelling of egg products. However, applying heat can impact the physical and functional characteristics of proteins as well as protein-protein interactions in the food system. Hence, adding sucrose is one of the alternative ways to not only improve the taste but also to stabilize the native structure of egg white, resulting in an increase in the thermal denaturation temperature (Mohammadi Nafchi et al., 2013). It can affect the gelling properties of egg white, which contributes to the food structure, texture, and stability (Kulmyrzaev et al.,

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[1]*Khon Kaen University, Faculty of Technology, Department of Food Technology, Khon Kaen, Thailand.*
*Corresponding author: suptha@kku.ac.th

2000). Tubtim Chumpae rice is a Thai red rice strain, RD69, which is hybridized between Hom Mali rice (Jasmine rice) and Sung Yod Patthalung rice. Previous studies showed that the effects of rice bran hydrolysate (RBH) have been revealed in hypertensive rats, *i.e.*, antioxidant and angiotensin-converting enzyme inhibitory activities, as well as improved endothelial function (Boonla et al., 2015). Recently, Senaphan et al. (2021) reported that RBH may possess cardio-protective effects against autonomic imbalances, cardiac oxidative stress, and structural changes in metabolic syndrome. RBH's excellent quality makes it a promising functional ingredient, but its bioactive compounds can be unstable in harsh environmental conditions, such as high temperature, oxygen exposure, and gastrointestinal environment, leading to degradation and decreased absorption by the body. To address this issue, a semi-solid form such as a food gel is a potential carrier that can stabilize thermosensitive bioactive compounds in thermally induced gelling systems (Jin et al., 2020).

According to the preliminary survey, the most profile product among aged people resided in Khon Kaen Province, Thailand, is the pudding or jelly-like product. On top of this, jelly products with 0.5% of RBH were favored by the participants. Therefore, it would be useful to design healthy jelly-like products that are made of easily digestible protein mixed with bioactive compounds, *i.e.*, rice bran hydrolysate. However, there is relatively limited information about employing aerated composite gel with egg white protein as a delivery system for bioactive compounds, specifically rice bran hydrolysate. As such, this study aimed to investigate the effects of egg white and sucrose levels on the texture and microstructure of the composite aerated gels as well as their release fate *in vitro* digestion and bio-accessibility of RBH compared to non-aerated gels.

2. Materials and method

2.1. Materials

Egg white albumen "Hen Egg Albumen High Gel" (87.96% protein and 0.38% fat) was obtained from Nive company in Holland. Protein and fat concentrations were determined by the Association of Official Analytical Chemists (AOAC) methods (AOAC, 2000). Refined κ-carrageenan "Acquagel GC-300" and purified konjac powder were bought from the Marcel Carrageenan company in the Philippines and Monkey King Food Co., LTD, respectively. Sodium bicarbonate and sucrose were purchased from a local supermarket (Tesco, Khon Kaen province, Thailand). Protease P6 (E.C. 3.4.21.62), a bacterial alkaline serine endopeptidase from *Bacillus licheniformis* and Protease GN, a bacterial neutral metallo endopeptidase from *Bacillus amyloliquefaciens* (DuPont™ Genencor® Science, USA) were purchased from Siam Victory Co., Ltd (Bangkok, Thailand).

2.2. Chemicals

Reagents and chemicals were of analytical grade and purchased from Sigma Aldrich, USA. Folin-ciocalteu's reagent was purchased from CARLO ERBA, England.

2.3. Aerated egg white-polysaccharide gel preparation

2.3.1. Thermal treatment of aerated gels

A fixed level of 0.5% (w/w) Konjac glucomannan (KG) was dispersed in distilled water followed by 0.5% (w/w) sodium bicarbonate (SBC) to act as a porogen added with sucrose (0 and 7.5% w/w) (Table 1). An egg white dispersion (0, 3, and 6% w/w) was prepared and poured into the mixture, and 0.25% (w/w) κ-carrageenan (κ-car) was added lastly. The pH values of the samples were around 7.4. Samples were then heated at 70°C for 5 min in a shaking water bath. Samples containing egg white protein (EWP) alone or with KG did not form a complete gel, so these samples were not chosen for study.

2.3.2. Formation of aerated egg white protein gels

After thermal treatment, samples were aerated at 60±5°C and the speed of 2,000 rpm for 30 s, using an ACE homogenizer AM-8 (NISSEI Ltd., Tokyo, Japan). Aerated gels were poured into food grade silicone molds of 2.5 cm length, 2.5 cm width, and 2.8 cm thickness. Samples then were stored at 4°C for 24 h.

Controls (non-aerated gels) were produced under the same thermal treatment and stored at 4°C for 24 h. Control gels were only prepared for the gas hold up analysis of aerated gels and the *in vitro* digestion study. All samples were equilibrated at 25°C for 30 min before every analysis. Experiments were conducted at least in triplicate.

2.4. Rice bran hydrolysate preparation

Tub Tim rice bran was received from Chum Phae Rice Research Center (Chum Phae, Khon Kaen). Rice bran was defatted using Hexane and produced through subcritical alkaline water (SAW) followed by enzymatic hydrolysis to obtain the final product called RBH (Kaewjumpol et al., 2018). Briefly, DRB by hexane were dispersed in distilled water at a ratio of 1:5 (w/v) and citric acid 1.5% (w/w) was added. RBH was extracted using mild-subcritical alkaline water condition (110°C, 60 min, pH 8.0). After centrifugation at 10,000 ´g, 15 min, the rice bran residues were mixed with distilled water again at the same ratio before being hydrolyzed with 0.5% Protease P6 at pH 8.0 at 55°C for 4 h and 0.5% Protease GN at pH 7-8, 55°C for 2h. After centrifugation at 10,000 ´g, 15 min, supernatants then were collected for freeze drying and stored at -30°C for further use. RBH contained 13.2% protein and a total phenolic content of 25.5 mg GAE.g^{-1}.

Table 1. Combination of ingredients used in composite aerated gels with and without sucrose addition.

Treatment[1]	Egg white protein (% w/w)	Sucrose (% w/w)
E0S0	0	0
E3S0	3	0
E6S0	6	0
E0S	0	7.5
E3S	3	7.5
E6S	6	7.5

[1]Other ingredients such as konjac glucomannan, kappa carrageenan, and sodium bicarbonate were fixed at 0.5, 0.25, and 0.5% (w/w), respectively.

2.5. Texture profile analysis

Texture Profile Analysis (TPA) of gels was performed by a texture analyzer (TA. XT Plus, Stable Micro Systems, Godalming, UK) equipped with a cylindrical probe (P/50) at a speed of 2 mm/s and 1 mm/s at a pre-test speed. A 50% strain was used to deform the sample from its original height, enabling a 10-s relaxation time between compression cycles with slight modification from Suebsaen et al. (2019). TPA parameters were calculated, *i.e.*, hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness.

2.6. Syneresis

Gel samples were removed from silicone molds after setting overnight at 4°C. They were weighed as day 0 and put in 50 ml centrifuge tubes for three days at 4°C. Gels were removed from the tubes and weighed again. Filter paper was used to blot water from the bottom of the tubes, since the amount of water that leaked out was very small. The percentage of syneresis was calculated using the following Equation 1 (Banerjee & Bhattacharya, 2011).

$$\% \text{ Syneresis } = [(m_0 - m_t)/m_0] \times 100 \quad (1)$$

Where:

m_0: weight of aerated gels at Day 0;

m_t: weight of aerated gels at Day 3.

2.7. Density and gas hold up of aerated egg white protein gels

The flotation method was applied to determine the density of the aerated gels following the method of (Zúñiga et al., 2011). Briefly, aerated gels were placed in a closed container filled with water at 30°C. The density was calculated as Equation 2:

$$\rho_{ag} = \frac{\rho_w \times m_{ag}}{m_{ag} + m_w - m_{w+ag}} \quad (2)$$

Where:

ρ_w: the density of water at 30°C (0.99567 g/cm^3);

m_{ag}: mass of aerated gels (g);

m_w: mass of the container filled with water alone (g);

m_{w+ag}: mass of the container with water and the aerated gels (g).

The gas-hold up capacity was determined by comparing the density of aerated gels (ρ_{cg}) with that of the control gels (ρ_{cg}) (Equation 3).

$$\emptyset = \left(1 - \frac{\rho_{ag}}{\rho_{cg}}\right) \times 100\% \quad (3)$$

2.8. Microstructural analysis

Aerated gels were cut into small pieces of 2-4 mm thickness and 4 x 4 mm of length and width, which were then frozen at -60°C for 1 day and freeze-dried (CHRIST, DELTA 2-24 LSC). The freeze-dried gels were fixed onto an aluminum Scanning Electron Microscopy (SEM) stub with the support of double-sided adhesive carbon tape, coated with a thin layer of gold using an EMITECH K500X Sputter Coater (Quorum Technologies Ltd, Laughton, East Sussex, UK). Eventually, they were imaged for microstructures using Scanning Electron Microscopy (HITACHI, S3000N, Japan). Sample micrographs were screened at various magnifications, and only 200 x magnification images were shown in this study.

2.9. In vitro *digestion procedure*

Sensory evaluation (data not shown) was performed to select an optimal treatment for further study *in vitro* digestion. Tubtim Chumphae RBH (RBH 0% and 1%) was employed as the model for a bioactive compound. The fabrication process of aerated gels incorporating RBH was the same as the method mentioned in section 2.3.2. RBH was added lastly before aeration. Prior to *in vitro* digestion, textural properties, syneresis of the aerated and non-aerated gels, as well as gas hold-up capacity of aerated gels were investigated following the conditions stated in 2.5, 2.6, and 2.7.

The *in vitro* digestion method used was adapted from (Liu et al., 2004) and (Seraglio et al., 2018). Before digestion, 2 g of aerated or non-aerated gel samples were blended and mixed with saline solution to obtain a final volume of 10 mL and subjected to heating at 37 ± 1°C in a shaking water bath at 95 rpm. The mixture was then acidified with 2 ml of porcine pepsin (40 mg in 1 mL 0.1 M HCl), incubated at 37±1°C and speed for 1h for gastric simulation. For pancreatic digestion, pH of the samples was increased to 7.4±0.1 with 0.9 M $NaHCO_3$ followed by the addition of 200 µL of bile salts (40 mg of glycodeoxycholate, 25 mg of taurodeoxycholate and 40 mg of taurocholate in 1 ml saline solution) and 250 µL pancreatin (80 mg in 1 mL saline solution), and the samples were incubated for another 2 h. Samples were centrifuged at 10,000 ´g for 5 min. The supernatants were kept at -18±2°C to quantify total phenolic, antioxidant activity, and protein contents.

After centrifugation, the compounds left in the supernatant were considered bioaccessible through gastrointestinal digestion. The ratio between the post-pancreatic concentration and the concentration before digestion without pepsin was determined as *in vitro* bioaccessibility (Helal et al., 2014).

2.9.1. Total phenolic content

Total phenolic content (TPC) was modified from (Kaewjumpol et al., 2018). Briefly, supernatant (200 µL) was mixed with 800 µL of Folin reagent (Folin: Distilled water, 1:10 v/v) and left at room temperature for 10 min. Then, 2 mL of 7.5% Na_2CO_3 was added and left in the dark at room temperature for 30 min. Finally, absorbance of 765 nm was measured using a UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Japan).

Gallic acid was employed as a standard and TPC was expressed in mg per 100 g dried weight of gel.

2.9.2. ABTS Assay

ABTS radical scavenging activity was determined according to Re et al. (1999), with some modifications. The ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS (7 mM) with 2.45 mM potassium persulfate at an ambient temperature for 12-16 hours in the dark. 0.1M Phosphate buffer, pH 7.4 was used to dilute an ABTS working solution to obtain an absorbance of 0.7 ± 0.2 at 734 nm, prior to assay. 100 µL of sample were mixed with 3 ml of the diluted ABTS solution and incubated in the dark at room temperature for 6 min. The ABTS radical scavenging activity was expressed as the Trolox equivalent antioxidant capacity (TEAC) in Trolox mg per 100 g dried weight of gel.

2.9.3. Protein content

Lowry's method was employed to measure the protein content following Fryer et al. (1986) using a microplate reader (SpectraMax M3, Molecular Devices, USA). Absorbance was recorded at 750 nm, and bovine serum albumin (BSA) was used as a protein standard and the result was expressed in BSA mg equivalent per 100 g as the dried basis of the gel.

2.10. Statistical analysis of data

Data were analyzed at a significant level of 0.05 by Analysis of Variance (ANOVA), using the SPSS software, version 23 (IBM software, NY, USA). Differences in mean values were evaluated by using Duncan's multiple range tests; $p<0.05$. An independent t-test was used to analyze the gas hold-up capacity of aerated gels with 0 and 1% of RBH. Data were recorded as means±standard deviations.

3. Results and discussion

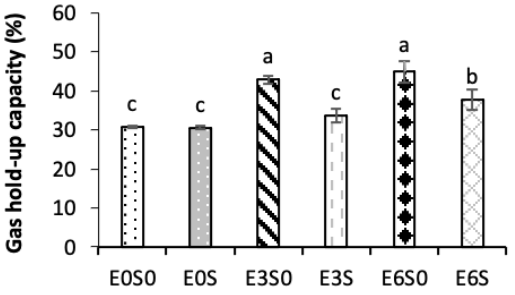
3.1. Texture profile analysis

A two-bite compression test was performed to evaluate the textural properties of aerated gels. Only EWP was found to have significant effect on the aerated gel texture ($p<0.05$). An increase in all textural properties was noticed at higher EWP concentrations (Table 2). The values of chewiness, gumminess, and springiness increased as hardness and adhesiveness values increased in proportion to EWP. Tomczyńska-Mleko et al. (2022) also found that the hardness of aerated gels increased along with protein concentration. Additionally, according to our preliminary study, the interaction between EWP and a mixture of κ-carrageenan and konjac glucomannan could enhance gel strength concomitance with other studies (Alavi et al., 2018; Huang et al., 2021; Tang et al., 2021; Wu et al., 2020). With decreasing EWP, gumminess and chewiness also declined due to low hardness.

Adding sucrose also improved all textural properties of the aerated gels, except for cohesiveness and springiness (Table 2). A study conducted by Yamul et al. (2013) on texture of whey protein gels with different sucrose content showed that sucrose increased hardness, cohesiveness, and adhesiveness of the gel and produced small pores. It can be considered that the attraction between protein molecules through hydrophobic interaction could be enhanced with the presence of sucrose.

3.2. Gas hold-up capacity

There was a significant interaction effect between EWP and sucrose ($p<0.05$) on the porosity of gels. It can be noticed that treatments with sucrose obtained lower gas hold-up capacity (Figure 1). Sucrose increased the viscosity of liquid mixtures, which made it difficult to incorporate air into the system resulting in a decrease in gel porosity; yet, its interfacial elasticity could help stabilize foams better (Raikos et al., 2007). Whereas treatments without EWP obtained the lowest gas hold-up capacity since there was no EWP to enhance foaming ability



E0: 0% egg white protein; E3: 3% egg white protein; E6: 6% egg white protein; S0: 0% sucrose; S: 7.5% sucrose; *different superscript letters (a-c) in each bar indicate significant differences ($p \leq 0.05$).

Figure 1. Gas hold-up capacity of aerated gels treated with different levels of egg white protein and sucrose*.

Table 2. Texture profiles of aerated gels with different egg white protein and sucrose concentrations*.

Egg White Protein[1] (%)	Hardness (g)	Adhesiveness (g. sec)	Cohesiveness	Springiness	Gumminess (g)	Chewiness (g)
0	1,003.11^{c}±208.10	-61.35^{a}±16.66	0.48^{c}±0.02	0.41^{c}± 0.02	486.58^{c}±101.70	200.19^{c} ±44.45
3	1,357.06^{b}±118.32	-129.26^{b}±25.82	0.54^{b}±0.01	0.47^{b}±0.01	735.38^{b}±66.20	348.56^{b}±34.14
6	1,569.86^{a}±146.39	-228.11^{c}±17.93	0.57^{a}±0.02	0.51^{a}±0.02	896.51^{a}±82.60	453.74^{a}±47.79
Sucrose[2] (%)						
0	1,212.93^{B}±112.64	-127.37^{B}±10.89	0.53^{A}±0.01	0.46^{A}±0.01	650.60^{B}±55.53	306.35^{B}±27.87
7.5	1,407.09^{A}±122.17	-151.77^{A}±19.15	0.54^{A}±0.02	0.47^{A}±0.02	761.71^{A}±60.48	361.97^{A}±31.43

*Data show mean±standard deviation of the 7 replicates; Different superscript letters (a-c), (A-B) within column indicate significant differences among treatments ($p \leq 0.05$); [1]each level of egg white protein treatment denotes the average values of both 0 and 7.5% sucrose; [2]each level of sucrose treatment denotes the average values of 0, 3, 6% egg white protein.

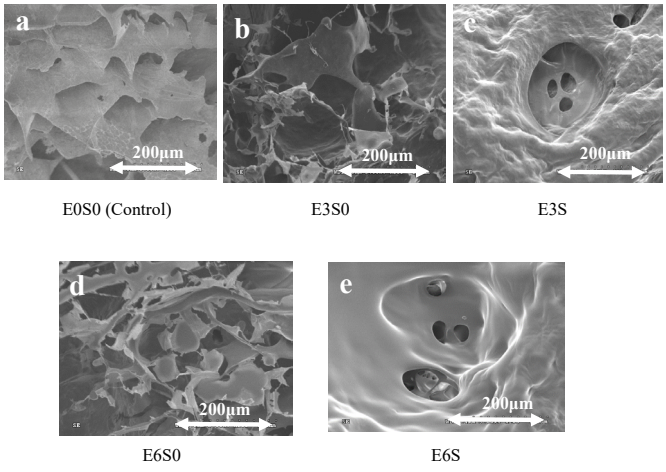
and stability due to the special amphiphilic nature of its proteins (Zúñiga et al., 2011). These results were supported by the SEM images shown in Figures 2A–2E.

3.3. Microstructure

SEM revealed that aeration somehow affected the structure of the gels. It was noticeable that a more heterogeneous and ruptured structure with large pore formation was seen in the treatments without sucrose, while smaller pores were found in treatments with sucrose. Sucrose could increase the denaturation time of proteins by protecting them against heat and altering the water structure, by enhancing hydrophobic interactions between proteins (Campbell et al., 2003). The viscosity of the continuous phase caused by sucrose could have prevented the bubbles from merging with each other (coalescence), since it could slow down the drainage of the continuous phase and caused lamella damage (Orrego et al., 2015). It can be seen in Figures 2C and 2E that there were still some small bubbles separated by the strong lamella found in the treatment with sucrose.

3.4. Syneresis

Syneresis was statistically affected by EWP ($p<0.05$), but not by sucrose. The highest EWP at 6% produced the lowest syneresis (Figure 3). Without EWP, the gels obtained an open network structure with very large pore formation, resulting in high syneresis. This result may be attributed to the stronger gel network of the protein-polysaccharide system and the ability of egg white to hold water by itself (Khemakhem et al., 2019).



E0: 0% egg white protein; E3: 3% egg white protein; E6: 6% egg white protein; S0: 0% sucrose; S: 7.5% sucrose.

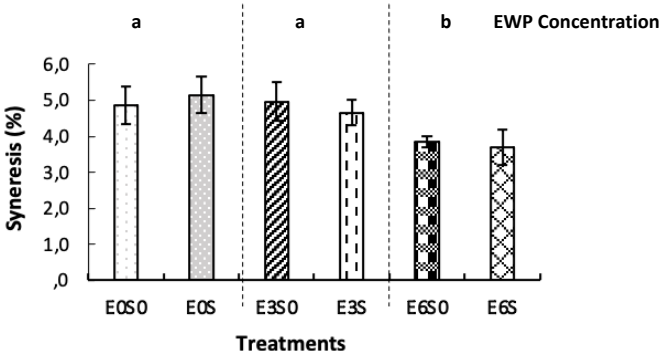
Figure 2. SEM microscopic images of egg white based aerated gel treated at different EWP and sucrose concentrations.

3.5. In vitro *digestion*

According to sensory evaluation (data not shown), EWP6% was not the most favorable for target participants aged 50 years old and older. They gave the highest overall liking score and acceptance for EWP 3% (w/w) and sucrose 7.5% (w/w) (E3S). The result of hardness obtained from TPA was negatively correlated with the liking score of texture attained in sensory evaluation. EWP 3% with sucrose exhibited the highest texture and overall liking scores closely located among attributes such as texture, color, and appearance of sensory attributes. Thus, it could be shown that the softer texture and lower whiteness of aerated gels were more attractive to panelists aged 50 years old up (Supplementar Figure 1). As a result, E3S was selected to add RBH at 0% and 1% (w/w) denoted as (A0 and ARB) and (C0 and CRB) for aerated gels and non-aerated gels with RBH 0% and 1%, respectively.

3.5.1. Texture profile analysis

RBH and aeration significantly increased gel hardness, cohesiveness, and springiness (Table 3). The protein in RBH could enhance the electrostatic interactions among polymers in the system in accordance with our previous EWP result. Furthermore, many studies reported the effect of phenolic compounds on protein cross-linking, thus altering protein functionality (Ozdal et al., 2013) and promoting gel texture.



E0: 0% egg white protein; E3: 3% egg white protein; E6: 6% egg white protein; S0: 0% sucrose; S: 7.5% sucrose; *different superscript letters (a-b) indicate significant differences ($p \leq 0.05$).

Figure 3. Effects of egg white concentration on syneresis of aerated gels*.

Table 3. Hardness, cohesiveness, springiness and syneresis of aerated and non-aerated gels with or without rice bran hydrolysate*.

Treatments[1]	Hardness (g)	Cohesiveness	Springiness	Syneresis (%)	Gas hold-up capacity (%)
C0	366.14^{c}±58.09	0.26^{c}±0.03	0.22^{c}±0.03	5.35^{a}±0.68	-
CRB	605.78^{b}±68.84	0.33^{b}±0.04	0.28^{b}±0.04	4.08^{b}±0.31	-
A0	1396.80^{a}±51.56	0.55^{a}±0.01	0.49^{a}±0.01	4.33^{b}±0.38	43.71^{b}±6.54
ARB	1576.05^{a}±136.32	0.56^{a}±0.01	0.50^{a} ±0.01	4.42^{b}±0.14	52.54^{a}±6.19

*Data show mean±standard deviation of the 6 replicates; different superscript letters (a-c) within column indicate significant differences among treatments ($p \leq 0.05$); [1]C0 and CRB: Non-aerated gels with RBH 0% and 1% w/w; A0 and ARB: aerated gels with RBH 0% and 1% w/w.

Moreover, aeration could swiftly unfold the protein structure and produce a cohesive protein layer (Tang et al., 2003).

3.5.2. Syneresis

Interaction between RBH and aeration showed a significant difference ($p<0.05$). Unsurprisingly, C0 obtained the highest syneresis (Table 3). The result was in line with the syneresis mentioned in section 3.4, as it obtained a fragile structure that could not retain water compared to aerated ones. Also, the addition of ARB could produce stronger gel networks that prevent water leakage.

3.5.3. Gas hold-up capacity

The capacity to obtain more gas was observed in ARB compared to A0 (52.54 *vs*. 43.71%) ($p<0.05$). As mentioned above, proteins and polyphenols in RBH and aeration interactively improve hardness, cohesiveness, and springiness of the gel (Table 3). Thus, the bubbles were probably well protected inside by the thicker and stronger lamella.

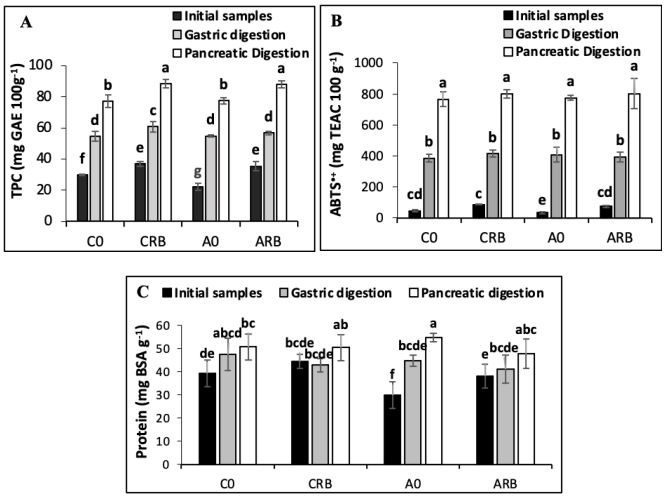
3.5.4. Total phenolic content, antioxidant activity and protein in vitro digestion

TPC, ABTS, and protein contents were significantly increased after digestion. Surprisingly, A0 obtained the lowest amount of TPC, ABTS, and protein among all treatments ($p<0.05$) (Figures 4A, 4B and 4C). It could be attributed to the mechanical force from aeration denaturing the proteins, leading to a greater exposure of the hydrophobic surfaces which can strongly bind with more hydrophobic phenolic compounds (Le Bourvellec & Renard, 2012). As a result, the bioaccessibility of A0 was the highest at 349.53 and 2,262.35% for TPC and ABTS, respectively (Table 4). When bioaccessibility values are higher than 100%, it indicates that the phenolic compounds in the food, particularly those with complicated structures, have been either metabolized or released from the food matrix (Santana Andrade et al., 2022). The result of TPC was also aligned with the ABTS result. After digestion, the antioxidant activity increased for all samples, probably as a result of the released antioxidant peptides and phenolic compounds derived from RBH, EWP and hydrocolloids.

During pancreatic digestion, protein content in all the treatments increased due to the activity of enzymes in the digestive system, in which proteins were digested into small peptides. Similarly, TPC of CRB increased as in line with other studies in which pepsin enhanced the release of phenolic compounds (Manach et al., 2004; Parada & Aguilera, 2007; Zhang et al., 2020). Tomczyńska-Mleko et al. (2014) also reported that air bubbles enlarged the contact surface with pepsin, which enabled ions to be released faster from aerated gels and resulted in a quicker hydrolysis.

4. Conclusion

In conclusion, egg white protein and sucrose mainly affected the aerated gel textural properties, syneresis, and gas hold-up capacity. EWP enhanced textural parameters, syneresis, and gas hold up capacity, while sucrose hindered the ability to create air bubbles. Adding RBH resulted in a stronger aerated gel network with the aid of phenolic-protein interactions, thus enhancing gas-hold up capacity and reducing syneresis. *In vitro* digestion, TPC, ABTS, and protein content were dramatically increased. Aerated gels obtained higher bioaccessibility than that of non-aerated gels, which could indicate that their bioactivity was more readily available for uptake into the gut and at the systemic level. Therefore, this study provides useful insights into designing new food matrices with a controlled release fate of nutrients *in vitro* digestion, which can affect the bio-accessibility of bioactive compounds.



C0: non-aerated gels (control) with RBH 0% w/w; CRB: non-aerated gels (control) with RBH 1% w/w; A0: aerated gels with RBH 0% w/w; ARB: aerated gels with RBH 1% w/w; GAE: Gallic acid equivalents (mg.100 g^{-1}); TEAC: Trolox equivalent antioxidant capacity (mg.100 g^{-1}); BSA: Bovine serum albumin equivalent (mg. g^{-1}); *Different lower-case letters (a-g) on each bar show significant differences ($p\leq0.05$).

Figure 4. (A) Total phenolic content (TPC) (A), (B) ABTS and (C) Protein content of C0, CRB, A0, and ARB before and after *in vitro* gastro-pancreatic digestion*.

Table 4. Bioaccessibility (%) of phenolic compounds of aerated and non-aerated gels with or without rice bran hydrolysate.

Treatments	Bioaccessibility (%)[1,2]			
	C0	CRB	A0	ARB
TPC	257.79	239.32	349.53	247.50
ABTS	1694.41	926.84	2262.35	1081.85

[1]Bioaccessibility (%) is the percentage ratio between the post-pancreatic concentration and the concentration before the digestion without pepsin; [2]C0 and CRB–Non-aerated gels (control) with RBH 0% and 1% w/w; A0: aerated gels with RBH 0% w/w; ARB: aerated gels with RBH 1% w/w; TPC: total phenolic content; ABTS: ABTS radical scavenging activity (The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation-based assay.

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