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Effects of Food Proteins on Sensory and Physico-Chemical Properties of Emulsified Pork Meatballs

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Abstract Emulsified pork meatballs (Kung-Yuan or meatballs) are common emulsified meat products in Chinese cultures and are widely popular among Taiwanese and Chinese consumers. In recent years, the concept of clean labels has become prominent, and consumers have started to demand products without artificial additives. In this study, we investigated the effects of food proteins-soy protein, sodium caseinate, whey protein, egg albumen powder, and skim milk powder on the texture and sensory acceptability of phosphate-free meatballs. The results were compared with the texture and sensory acceptability of meatballs with phosphate. Texture analyses revealed that adding different food proteins to phosphate-free meatballs increased their hardness. Among them, meatballs with egg albumen powder were the hardest, followed by those with sodium caseinate and those with whey protein. Meatballs with phosphate and phosphate-free meatballs without any protein additive were less chewy than meatballs with any of the five protein additives, adding the egg albumen powder, sodium caseinate, and whey protein, however, could increase the meatballs' chewiness. Quality analyses showed that all groups of meatballs were had pH values between 6.36 and 6.96 and had water compositions between 49.81% and 56.79%. Sensory evaluation revealed that soy protein had negative effects on sensory acceptance, whereas whey protein scored the highest in overall sensory evaluation. The composition analysis results of meatballs with whey protein and phosphate-free meatballs exhibited no significant differences. The hygiene quality test showed that the total plate count of all meatball groups were had less than 10^5 CFU/mL and *Escherichia coli* and coliforms weren't detected. In summary, although adding soy protein negatively influenced the meatballs' sensory perceptions, all other types of food protein additives improved the texture of phosphate-free meatballs. The sensory evaluation indicated that adding whey protein is the most effective way to improve a meatball's texture. The sensory evaluation results indicated that the overall acceptance of meatballs with whey protein was not significantly different from that of meatballs with phosphate.

Keywords: *meat products, meatballs, kung-wan, phosphate, protein*

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1. Introduction

Clean labels are a trend in the food industry and have captured consumers' attention. During the 1980s, the European Union introduced E numbers for food packaging. Food with fewer E numbers tended to be cleaner [1]. In recent years, increasingly, many studies have investigated clean labels [1], reflecting the interest in this topic. To date, Concept of clean label has not been clearly defined. In order to meet the requirements of sanitation, safety, and regulations and consider health, nutrition, sensory acceptance, and shelf life, manufacturers have tended to employ natural additives instead of artificial additives to increase consumer acceptance and willingness to purchase processed products.

Meatballs are common emulsified meat products in Chinese cultures. Compared with western meatballs, Chinese meatballs are springier [2] and are therefore favored by Taiwanese and Chinese consumers [3]. Meatballs are composed mainly of pork ham and back fat, and they contain additives such as phosphates, salt, and seasonings. Studies have demonstrated that approximately 14% of daily salt intake is derived from meat products [4,5]. Due to the rise of clean labels and consumers' increasing appeals for foods that are natural, healthy, less-processed, and low in additives, increasingly, many studies have investigated low-fat meatballs with few artificial additives [3,6]. For example, in some studies, the contents of artificial nitrates and phosphates in meatballs were reduced. The main roles phosphates play in meat products are to increase the extraction of salt-soluble proteins and enhance water retention [7,8]. Reduction of phosphates in meat products will reduce salt-soluble protein extraction and thus decrease the crosslinks between meats, leading to a decrease in the quality of the meat product. In response, studies have experimented with natural additives such as tapioca starch, carrageenan, whey protein, porcine blood plasma, and wheat bran to increase the quality of meat products [9,10,11,12].

In this study, we investigated meatballs in which phosphates were substituted with food proteins, namely soy protein, sodium caseinate, whey protein, egg albumen powder, and skim milk powder, and investigated their effect on the texture and sensory acceptability of phosphate-free meatballs. The results were compared with the texture and sensory acceptability of meatballs with phosphate.

2. Materials and Methods

2.1. Preparation of Meatballs (Kung-Yuan)

The meatballs were manufactured according to a traditional formula for Taiwan-style meatballs (Kung-Yuan): 85% Fresh pork ham (Cha I Shan Foods Co., Ltd, Taiwan) and 15% pork back fat (Cha I Shan Foods Co., Ltd, Taiwan) were mixed with 1.8% salt (99.5%, Taiyen Biotech Co., Ltd, Taiwan), 3.0% sugar (99.5%, Taiwan Sugar Co., Taiwan), 0.3% spices (Tomax Enterprise Co., Ltd, Taiwan), and 2% corn starch (LINCO Enterprise Co., Ltd, Taiwan). The tissues were ground with a meat chopper fitted with a plate of 20 mm diameter holes. The ground meat was packaged in plastic bags, 1.0 kg each, and stored at -18°C. Before use, the meats were maintained 20 h at 0°C and then they were mixed with the condiments and commercial whey protein(99.5%, Yihyuan Co., Taiwan), skim milk powder, sodium caseinate (99.5%, Gemfont Co., Taiwan), egg albumin (99.5%, Gemfont Co., Taiwan) and isolated soy protein(99.5%, Yihyuan Co., Taiwan). The sample was manufactured with sodium phosphate was considered as positive control and without sodium phosphate as negative control. As shown in Table 1, the experimental design consisted of two controls and five formulaes. After meatballs were boiled at 85°C until an internal temperature of 72°C, they were cooled and packaged in plastic bags (NY/LLDPE laminated film). All sample(0, 400, and 800 ppm) were measured at 3 day intervals and stored for up to 15 days at 4°C and on days 0, 30 and 90 of storage at -20°C.

Table 1. The Abbreviation of Different Treatments

Abbreviation	Treatment
WP	Add 2% whey protein
SM	Add 4% skim milk powder
SC	Add 4% sodium caseinate
EA	Add 6% egg albumin
ISP	Add 6% isolated soy protein
PC	Add 0.3% sodium phosphate
NC	Without any food additives

2.2. Texture Profile Analyses

Frozen meatballs were placed in a cooler (2°C) for approximately 12h. Once thawed, meatballs were heated at 100°C water bath for 7 min and then cooled to room temperature. For TPA measurement, the meatballs were cut by two sides to get a 20 mm depth strip. The texture profile analyses (TPA) indices of meatballs were determined using a texture analyzer (Model TA-XT2 Texture Analysis, England). The conditions of texture analyzer were modified by [13]. Pre-test speed: 2.0 mm/s; test speed: 2.0 mm/s; post-test speed: 2.0 mm / s; distance: 10.0 mm; time: 5.0s; trigger type: auto; and trigger force, 10 g.

2.3. Physicochemical Properties of Meatballs

The pH value of the meatballs was measured after homogenization (Interscience Co., Model BagMixer® 400P, France) with distilled water at a ration of 1:10 using a pH meter (Denver Instrument, USA). Moisture content was measured by the weight difference before and after oven drying at 105°C for 16 h. Ash content was measured by AOAC, 1970 method [14]. Saturated fat and trans fat was measured by GC method [15]. Crude lipid content was measured by drying the sample in a 105°C oven for 6 h and then extracting the lipid with ether in a Soxhlet extractor for 4 h. Crude protein content was measured by the Kjeldahl method (AOAC, 1984). The phosphate content measured by spectrophotometric ammonium molybdate method (AOAC, 1990) was based on using sulfuric acid to hydrolyze all phosphates into orthophosphates and measured at 690 nm with a spectrophotometer. Therefore, phosphate contents of the meatball samples were determined in terms of orthophosphates [7].

2.4. Sensory Analysis

The sensory analysis of meatballs was evaluated by 30 untrained assessors selected according to their habits. Samples were labeled with 3-digit random numbers and served in random order to assessor in individual booths. Assessors were instructed to cleanse their palates with water between samples. A hedonic test was carried out using 9 point scales (9 = like extremely and 1 = dislike extremely) in which the assessors evaluated different attributes: appearance, taste, texture, flavor, overall acceptability [16].

2.5. Microbial Status of Meatballs

The total plate count of meatballs were determined using 3 M PetrifilmTM (3M Co., USA) Aerobic Count Plate (AOAC OMA: 990.12) [17]. Plates were incubated at 37°C for 48 hour. Results were expressed as log10 CFU/g meatballs.

Coliform and E. coli of meatballs were determined using 3 M PeterifilmTM (3M Co., USA) E. coli and Coliform Count Plate (AOAC OMA: 991.14) [18]. Plate were incubated at 37°C for 24 hour. Results were expressed as log10 CFU / g meatballs.

2.6. Statistical Analysis

Data were analyzed using SPSS 12.0 for one-way ANOVA. Duncan's new multiple range test was used to resolve the difference among treatment means. A value of $p < 0.05$ was used to indicate significant difference.

3. Results and Discussion

3.1. The Texture Properties of Meatballs

Texture analysis was an indicator for meatball quality. Table 2 shows the results from texture property analysis (TPA). The analysis of meatball hardness indicated that positive and negative control groups scored lower. Including different additives evidently increased the meatballs' hardness: the 6% egg albumin (EA) treatment had the strongest effect, followed by the 4% sodium caseinate (SC) and 2% whey protein (WP) treatments. These results were similar to those of Hsu and Sun [3]. Adding egg albumen powder and sodium caseinate gave the meatballs a crispier texture, thereby increasing their hardness. The springiness and cohesiveness results were similar: the meatballs with the WP and 4% skim milk powder (SM) treatments ranked higher in these two categories because of excellent hydration and gelatinization. Thus, whey protein could decrease the hardness in meatballs and increase their springiness [19,20]. Hsu and Sun [3] indicated that adding whey protein made meatballs stickier and that gumminess and chewiness displayed similar trends to that of hardness. In the gumminess and chewiness results, the 0.3% sodium phosphate (PC) and no food additives (NC) treatments ranked lower, whereas the EA, SC, and WP treatments increased meatballs' gumminess and chewiness. Therefore, the harder a meatball, the chewier it is. Texture analysis indicated that the PC and NC treatments yielded softer and less chewy meatballs. Additives can effectively improve a product's texture [21,22] align it with consumers' preferences [3,23]. Thus, the PC and NC treatments created softer meatballs. Among the treatments, EA, SC, and WP improved meatballs' texture and yielded more favorable results by increasing meatballs' hardness and chewiness, thereby enhancing their acceptability to consumers.

3.2. The Physical Characteristics of Meatballs

Table 3 reveals that all the pH values for all groups were between approximately 6.36 and 6.96. The samples had water content between approximately 49.81% and 56.79%. Atughone et al. [24] indicated that heat treatment increased the hydration and plasticizing capacity of nonmeat additives, resulting in an increased pH in uncooked meatballs. The meatballs with the PC and 6% isolated soy protein (ISP) treatments had the highest pH values, possibly because phosphates have stronger hydration abilities [7,25], whereas soy protein has stronger water absorption properties [26], resulting in meatballs with higher water content. The meatballs with PC and SC treatments contained the highest ash content, followed by those with the SM treatment. The PC and SC treated meatballs contained sodium, whereas SM treated ones were less purified and contained more minerals. Therefore, meatballs with PC, SC, and SM treatments contained higher ash content, whereas meatballs with other treatments exhibited no significant differences. Phosphate was only detected in meatballs with PC treatment. Because the treatments of the other groups did not involve phosphate, phosphate was not detected in those meatballs.

3.3. The Microbial Status of Meatballs

Figure 1 illustrates meatball hygiene under different treatments. The results showed that *E. coli* and coliforms were not found in any meatballs. The Total Viable Count (TVC) in each group was less than 105, although some groups contained more bacteria than others. The TVCs of the meatballs with the WP and ISP treatments were lower than that of the positive control group. The TVC of the meatballs with EA treatment was the lowest, whereas those of the meatballs with SC and SM treatments were slightly higher than that of the positive control group.

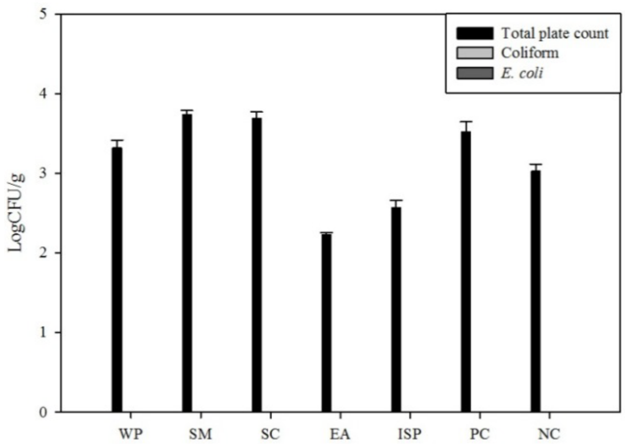

Figure 1. The Microbial Status of Meatballs Added Different Kinds of Food Proteins

Table 2. The Texture Properties (TPA) of Meatballs Added Different Kinds of Food Proteins (Mean ± SD, n = 15)

Treatment	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)
WP	1578 ± 239^{c}	0.92 ± 0.01^{ab}	0.76 ± 0.01^{a}	1208 ± 177^{bc}	1105 ± 145^{bc}
SM	1479 ± 194^{cd}	0.92 ± 0.01^{bc}	0.75 ± 0.02^{b}	1152 ± 180^{cd}	1058 ± 169^{cd}
SC	1829 ± 304^{b}	0.90 ± 0.01^{d}	0.73 ± 0.02^{c}	1312 ± 193^{b}	1180 ± 159^{b}
EA	2374 ± 307^{a}	0.93 ± 0.01^{a}	0.77 ± 0.01^{a}	1806 ± 209^{a}	1670 ± 186^{a}
ISP	1626 ± 137^{c}	0.90 ± 0.01^{d}	0.71 ± 0.02^{d}	1153 ± 117^{cd}	1037 ± 109^{cd}
PC	1379 ± 183^{d}	0.91 ± 0.02^{c}	0.76 ± 0.03^{a}	1052 ± 137^{d}	958 ± 130^{d}
NC	1505 ± 117^{cd}	0.92 ± 0.01^{abc}	0.75 ± 0.01^{b}	1128 ± 80^{cd}	1034 ± 76^{cd}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 3. The Physical Characteristics of Meatballs Added Different Kinds of Food Proteins (Mean ± SD, n = 3)

Treatment	pH value	Water Content (%)	Ash (%)	Phosphate (%)
WP	6.56 ± 0.01^{b}	53.73 ± 0.01^{b}	0.88 ± 0.01^{c}	N.D.
SM	6.53 ± 0.01^{b}	50.99 ± 0.01^{c}	0.96 ± 0.01^{b}	N.D.
SC	6.96 ± 0.01^{a}	51.68 ± 0.01^{c}	1.13 ± 0.01^{a}	N.D.
EA	6.49 ± 0.01^{b}	49.81 ± 0.01^{c}	0.88 ± 0.01^{c}	N.D.
ISP	6.85 ± 0.01^{a}	55.15 ± 0.01^{a}	0.91 ± 0.01^{c}	N.D.
PC	6.58 ± 0.01^{b}	56.79 ± 0.01^{a}	1.35 ± 0.01^{a}	0.18 ± 0.01
NC	6.36 ± 0.01^{b}	52.97 ± 0.01^{b}	0.87 ± 0.01^{c}	N.D.

N.D. means not detected.
Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 4. The Sensory Evaluation of Meatballs Added Different Kinds of Food Proteins (Mean ± SD, n = 30)

Treatment	Appearance	Taste	Flavour	Texture	Overall
WP	6.5 ± 1.2^{a}	7.0 ± 1.2^{a}	6.9 ± 1.3^{a}	7.0 ± 1.3^{a}	7.2 ± 1.2^{a}
SM	6.1 ± 1.5^{a}	6.4 ±1.4^{ab}	6.4 ± 1.4^{ab}	6.5 ±1.4^{ab}	6.4 ±1.5^{bc}
SC	6.5 ± 1.3^{a}	6.5 ± 1.1^{ab}	6.4 ± 1.2^{ab}	6.5 ± 1.3^{ab}	6.4 ± 1.2^{bc}
EA	6.4 ± 1.1^{a}	5.1 ± 1.7^{c}	4.7 ± 1.7^{c}	5.9 ± 1.5^{b}	4.8 ± 1.9^{d}
ISP	4.8 ± 1.6^{b}	4.7 ± 1.6^{c}	4.6 ± 1.7^{c}	3.3 ± 1.5^{c}	3.5 ± 1.6^{e}
PC	6.5 ± 0.6^{a}	6.7 ± 0.7^{a}	6.8 ± 1.4^{ab}	6.7 ± 1.4^{a}	6.9 ± 1.3^{ab}
NC	6.1 ± 0.7^{a}	6.0 ± 0.6^{b}	6.1 ± 1.4^{b}	5.8 ± 1.5^{b}	5.8 ± 1.5^{c}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 5. The Proximate Composition of Meatballs Added Different Kinds of Food Proteins (Mean ± SD, n = 3)

Treatment	Water Content (%)	Protein (%)	Fat (%)	Saturated Fat (%)	Trans Fat (%)	Ash (%)	Carbohydrate (%)
WP	53.73 ± 0.01^{a}	16.04 ± 0.01^{a}	29.48 ± 0.01^{a}	10.33 ± 0.01^{a}	0.07 ± 0.01^{a}	0.88 ± 0.01^{a}	0.75
NC	52.97 ± 0.01^{a}	15.74 ± 0.00^{a}	29.79 ± 0.01^{a}	10.46 ± 0.01^{a}	0.06 ± 0.01^{a}	0.87 ± 0.01^{a}	0.63

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

3.4. The Sensory Evaluation of Meatballs

The sensory evaluation reflected consumers' degrees of satisfaction. Meatballs with the ISP treatment ranked lower in appearance, but other groups exhibited no significant differences in their appearances (Table 4). Additives did not reduce consumers' favor for meatballs; in fact, they made meatballs more favorable than those with PC treatment. Meatballs with ISP treatment received the lowest score in the sensory evaluation, which was even lower than that of the negative control group, possibly because the soy protein notably altered meatballs' flavor, despite improving their texture. Consequently, consumers found them less acceptable [3,27]. Lee et al. [26] indicated that adding <1% of soy protein mitigated impairments to the flavor, texture, color, and overall acceptability of emulsified sausages. Although meatballs with the EA treatment scored highly in the TPA results, their acceptance among consumers was not correspondingly positive, possibly because the EA treatment increased the meatballs' hardness and chewiness. However, Hsu and Chung [2] noted that, although harder meatballs were more favored by consumers, excessive hardness decreased meatballs' overall acceptance [28]. Consequently, the meatballs with the EA treatment scored poorly in the sensory evaluation. Meatballs with the WP treatment had the most favorable results, which were even superior to those of the PC treatment. Therefore, the WP treatment would not affect meatballs' texture, and WP treated meatballs are suitable for consumers' preferences.

3.5. The Proximate Composition of Meatballs Added Within Whey Protein

The sensory evaluation results revealed that meatballs with the WP treatment had similar or higher consumer acceptance compared with meatballs treated with PC. To investigate whether those two meatballs groups differed in their basic compositions, we analyzed their proximate compositions and found no significant differences (Table 5). Bishop [29] mentioned that water-to-protein ratio affects meatballs' juiciness and tenderness. The higher this ratio is, the juicier and more tender the meatballs are. In the present study, meatballs with the WP and NC treatments contained similar water-to-protein ratios. Therefore, applying the WP treatment to meatballs does not decrease their juiciness or tenderness. In fact, these meatballs scored even higher in consumers' sensory evaluation. Hsu and Sun [3] specified that adding additives at <4% does not significantly affect the crude protein content in meat products. Therefore, the addition of 2% whey protein in this study did not substantially affect the basic composition of the meatballs.

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