Differences in Functional Properties of Mungbean Protein Concentrate and the Effect of Incorporation into Fish Sausages

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ABSTRAK

Sifat fisiko-kimia dan sifat berfungsi pekatan protein kacang hijau yang dihasilkan melalui (i) mendakan menggunakan kalsium sulfat dan (ii) mendakan menggunakan titik isoelektrik, bila dibandingkan, mengandungi 21.6 dan 67.3% protein masing-masing. Kelarutan pekatan pekatan tersebut berkait secara positif dengan pH dalam banjaran nilai pH 4-7. Kebolehan membusar berkait rapat dengan percentage of N larut ($r^2 = 0.98$) dan pH ($r^2 = 0.88$) sementara kestabilan busar ada kaitan dengan kehidrofilikan ($r^2 = 0.98$) pekatan. Semua pekatan dapat mengurangkan kehilangan berat, kekecutan dan meningkatkan ketegasan sosej ikan yang telah di masak. Peratus kehilangan berat dan kekecutan berkait secara negatif dengan peratus protein larut, pH dan sifat kebolehan membusar pekatan. Sifat-sifat berfungsi pekatan-pekatan tersebut, bila ditambah kedalam sosej ikan pada kandungan 1-2% akan mempengaruhi tekstur produk tersebut. Dalam penilaian deria, sosej ikan yang mengandungi protein-protein tumbuhan mendapat skor yang lebih tinggi untuk penerimaan keseluruhan, walaupun tiada perbezaan bererti dalam skor cita-rasa atau tekstur dan skor yang lebih rendah didapati untuk kesarian produk bila dibandingkan dengan hasil kawalan.

ABSTRACT

The physico-chemical and functional properties of mungbean protein concentrate prepared by (i) calcium sulphate precipitation (MBC-Ca) and (ii) isoelectric point precipitation (MBC-pI) containing 21.6 and 67.3% protein respectively, were compared. The solubility of the concentrates was positively correlated with pH within the range of 4-7. The foaming ability was closely correlated with percentage of soluble N ($r^2 = 0.98$) and pH ($r^2 = 0.88$) while the foam stability was correlated with the hydrophilicity ($r^2 = 0.98$) of the concentrates. All concentrates were able to reduce the weight loss, shrinkage and increase the firmness of cooked fish sausages. The weight loss and shrinkage were negatively correlated with the soluble protein, pH and foaming ability of the concentrates. The functional properties of the concentrates, when added at a level of 1-2%, influenced the texture of the fish sausages. In organoleptic evaluations, fish sausages incorporating the plant proteins scored higher for overall acceptability, even though there was no significant difference in flavour or texture and a decrease in juiciness of the product compared to the control.

INTRODUCTION

Mungbean (*Phaseolus aureus*) contains about 20-27% protein and has an amino acid profile comparable to soybean (Evans and Bandermer 1967; Fan and Sosulski 1974; Thompson *et al.* 1976). It is an important protein source in India, and partially replaces some of the ingredients for baby foods, snacks and noodles in the Philippines, China and Japan (Bhumiratana and

Nondasuta 1972). However, its colour and 'beany' flavour limit its use, unless it is dehulled or converted to protein concentrate. Mungbean protein concentrate (MBC) can be a by-product of mungbean noodle factories, which only make use of the starch. MBC may not only improve the nutritional content but also the flavour, texture and appearance of the food.

The quality of MBC has been shown to be comparable to soy protein concentrate (SPC) (Thompson 1975; Bhumiratana 1977). Studies on MBC have been limited to its use as a meat analogue (Narayana and Narasinga Rao 1982). Its functional properties have not been extensively studied with regard to its usefulness as a stabilizer, thickener, milk substitute, emulsifier, extender and binder in various products. The functional properties of MBC merit research for developing its use in food, especially in cereal-based products because of the complimentary amino acid pattern. This work was carried out to compare selected functional properties of MBC prepared by two different methods and the effect of incorporating it in a product such as fish sausage.

MATERIALS AND METHODS

Dehulled mungbeans from Thailand of unknown storage history were obtained from retail shops near the university. Foreign particles and spoiled beans were removed. Mungbean flour (MBF) was prepared by grinding the beans in a hammer mill to pass 710 nm mesh size sieve (U.S. standard mesh). Commercial grade soya protein concentrate from defatted soya flour (Marksaids Malaysia) was used for comparison (standard) of the functional properties of prepared mung bean concentrate (MBC). Fresh bighead carp (*Aristichthys nobilis*), obtained from Salak South, Kuala Lumpur, were harvested at 6-9 months maturity (about 45-55 cm long and 1.8-2.5 kg weight). Unless otherwise stated, all experiments were carried out at room temperature (30 ± 3°C). Preparation of MBC was carried out in 6 replicates.

Calcium-precipitated MBC (MBC-Ca) was prepared by extracting MBF with about 5 times its weight of water and filtering through a muslin cloth. The extracts were brought to 90°C, cooled to 85°C, allowed to precipitate for about 30 min with 0.4% (w/w) $CaSO_4$ (BDH Chemical Ltd, Poole, England), centrifuged and dried at 45°C for 24 h before grinding and sieving (280nm sieve) (Payuma *et al.* 1985).

Isoelectric point-precipitated MBC (MBC-pI) was precipitated from similar aqueous extracts of MBF by adjusting the pH down to the isoelectric point of the mungbean protein (pH 4.0) with 6N HCl (Analytical grade, Gruppo Montedison, Farmatalia CaHo Erba), centrifuging and drying as above (Chang and Satterlee 1979).

The percentage of soluble nitrogen of the protein was determined by the Biuret method using bovine serum albumin (Sigma, B2518 lot No33H6780) as the standard (Layne 1957; Narayana and Narasinga Rao 1982). Emulsifying ability was determined by dropping RBD palm olein at 0.2 ml/s into a continuously stirred (Magnetic stirrer) suspension of 2.0 g MBC in 23 ml distilled water (Lin *et al.* 1974). The end point was when the ammeter needle (Sanwa, YX-360 TR, Taiwan) suddenly showed a change in reading and the emulsion separated into two phases.

The foaming ability (Lawhon *et al.* 1972) was the foam volume at 30 s after homogenizing 100 ml, 1.0% aqueous suspension of MBC for 5 min using a Kinematica emulsifier (Switzerland) as measured using a 250-ml measuring cylinder. The volume of the foam was monitored every 5 min for 120 min. The foam stability was calculated by the formula $(2t/50V_m)$ where V_m = max foam volume (ml), and t = time in min for the foam to collapse to $V_m/2$ (Townsend and Nakai 1983).

Fat-holding capacity (Lin *et al.* 1974) was determined by measuring the free oil remaining after mixing 0.5g MBC to 5.0 ml of RBD palm olein for 30 s, standing for a further 30 min, and then centrifuging for 25

min at 1750 g. Colour was determined using the Hunter-lab colorimeter (model D25, USA) with a white tile (a = -0.9, b = 0.5 and L = 91.25) as the standard. pH was measured using a pH meter (Jenway, Model PHM64). Moisture was determined by drying 2.0 g MBC *et* 105°C to constant weight (AOAC 1980). Crude protein was determined on 0.15 g MBC using the micro-Kjeldahl method where crude protein = N × 6.25 (AOAC 1980).

Preparation of Fish Sausage

Fish sausage was prepared by mixing 50g minced fish flesh, 50g tapioca flour and 1g salt. The proximate composition of the fish sausage was 16.4% protein, 4.3% fat, 76.9% moisture and 1.2% ash. Protein concentrate was added to minced fish meat at 0, 1.0, 1.5 and 2.0% level in the presence of 1% NaCl. They were mixed in a Kenwood Chef mixer (model A901) at a speed of 4 rev/s for 5 min at room temperature, then inserted into 35-mm diameter cellulose casing and tied at the ends. After labelling, the sausages were kept frozen (at -20°C) for 24 h to allow for the formation of intermolecular crosslinks.

Analysis of the Fish Sausage

Frozen fish sausage was thawed at 30°C for 30 min, boiled for 25 min, then air cooled for 15 min.

The weight loss and shrinkage were determined by comparing the average weight and circumference of six sausages before and after boiling.

The texture of the sausage was empirically determined using an Instron Universal testing machine (Model 1140) with a 5-cm long puncture probe on thawed sausage samples at a crosshead speed of 50 mm/min, a 5 kg load cell and noting the yield/breaking stress.

The cooked fish sausages were organoleptically evaluated by 10 trained panellists

for colour, flavour, texture, juiciness and overall acceptability on a 1-7 hedonic scale (7 = extremely like; 1 = extremely dislike) (Larmond 1982). The data were analysed using multiple-range test (Walpole 1982) and regression analysis.

RESULTS AND DISCUSSION

The protein content of both MBCs was found to be lower than that of SPC (Table 1). The solubility of the protein was positively correlated ($r^2 = 0.83$) with pH within the range studied independently of how they were prepared. Therefore, theoretically the solubility could be adjusted as required by bringing the pH away from the isoelectric point of the protein and increasing the net charge.

Regression analysis shows that the amounts of moisture in the MBCs and SPC after preparation were positively correlated with the percentage of soluble crude protein of the concentrates ($r^2 = 0.99$), indicating that the hydrophilic nature of the soluble protein has some influence on the moisture content of the concentrates.

Emulsifying Capacity and Fat Absorption Capacity

For all concentrates, the values of fat absorption capacity and emulsifying capacity followed a similar trend; the highest value was seen in MBC-pI and the lowest value in MBC-Ca (Table 1). Voutsinas and Nakai (1983) showed that a close relationship exists between fat binding capacity and surface hydrophobicity, while Kato and Nakai (1980) found a significant correlation ($P < 0.01$) between the emulsifying capacity and the hydrophobicity of proteins. The present study confirms the close relationship between fat-binding capacity and emulsion capacity, and that they most likely relate to the surface hydrophobicity of the protein molecules. The result above thus indicates that the

TABLE 1

Physico-chemical properties of calcium sulphate precipitated mungbean protein concentrate [MBC-Ca], isoelectric point precipitated mungbean protein concentrate [MBC-pI] and soya protein concentrate [SPC]

Sample	MBC-Ca	MBC-pI	SPC	MBF (mungbean flour)
crude protein (%)	$51.6^c \pm 0.6$	$67.3^b \pm 4.1$	$77.1^a \pm 2.2$	$22.3^d \pm 1.6$
moisture (%)	$10.35^b \pm 10.05$	$10.18^b \pm 0.14$	$12.08^a \pm 0.05$	
soluble N* (%)	$2.6^c \pm 0.7$	$2.1^c \pm 0.5$	$6.3^a \pm 0.4$	$3.6^b \pm 0.3$
pH	$5.71^b \pm 0.01$	$4.06^c \pm 0.04$	$7.01^a \pm 0.003$	6.25
fat absorption (g oil/g protein)	$1.9^a \pm 0.3$	$2.4^a \pm 0.7$	$2.3^a \pm 0.6$	
emulsifying capacity (g oil/g protein)	$15.2^c \pm 0.6$	$30.2^a \pm 10.8$	$21.7^b \pm 0.4$	
foaming ability (ml)				
0.5 min	12.0	3.9	36.0	
5.0 min	7.3	3.9	36.0	
120 min	4.3	0.5	18.7	
Best fitted line				
Y =	6.84–0.016x	3.015–0.023x	36.17–0.145x	
$r^2 =$	0.74	0.85	0.99	
rate of foam collapse (ml/min)	0.02	0.02	0.15	
foam stability $(2t/50V_m)$	0.17	0.46	0.14	
t = (min) time to reach $V_m/2$	50	45	125	
colour				
(L) lightness	$84.88^a \pm 0.05$	$84.40^b \pm 0.03$	$82.35^c \pm 0.03$	
(a) + red, -green	$-0.72^a \pm 0.08$	$-2.15^b \pm 0.03$	$-1.99^c \pm 0.05$	
(b) + yellow, -blue	$12.77^a \pm 0.19$	$18.05^b \pm 0.03$	$30.38^c \pm 0.11$	

* = pH unadjusted

Means within the same row followed by the same letter are not significantly different (p > 0.05). The standard deviations were calculated from at least 6 replicates

surface hydrophobicity of MBC-pI is much greater than of MBC-Ca. At the isoelectric point, the electrostatic charges on the randomly coiled heated protein molecule interact, causing exposure of more hydrophobic side chains (Bigelow 1967) and protein insolubility.

However, there was poor correlation between emulsifying capacity or fat absorption and protein solubility. This agrees with the work of Wang and Kinsella (1976) who found no statistical correlation between emulsifying capacity and protein solubility, but a high correlation ($r^2 = 0.8$) between emulsion stability and protein solubi-

lity. The emulsifying properties of proteins ultimately depend on the hydrophile:lipophile balance, and do not necessarily increase as the proteins become more hydrophobic (Rand 1976). The ability of proteins to bind lipids is important for applications such as meat replacers and extenders.

In this study, under the same rates of blending, oil addition and temperature, the fat absorption capacity and emulsion capacity were found to be correlated only with the protein content in the concentrates ($r^2 = 0.87$ and 0.52 respectively) and not with pH.

Foaming Ability

The foaming ability was very closely correlated with the percentage of soluble N ($r^2 = 0.98$), and also with pH of the concentrate ($r^2 = 0.88$), as Cheftel *et al.* (1985) and Townsend and Nakai (1983) respectively had also found. The availability of mixtures of acidic and basic proteins (opposite charges) is thought to be important for the inter-molecular electrostatic interactions and strength of the 'skin' around the air bubbles (Hart 1986).

Foaming ability was found not to be related to emulsion capacity or fat absorption capacity, but surface hydrophobicity, which confirms the work of Townsend and Nakai (1983). The presence of salt in the final product (as with MBC-Ca and MBC-pI preparation) also reduces foam capacity and stability (Graham and Phillips 1976), but increases emulsion capacity (Wang and Kinsella 1976) due to the unfolding of the protein. The salt content or ionic strength of the MBCs was not determined in this work.

Foam Stability

Rate of foam collapse was found to be closely correlated ($r^2 = 0.98$) to the water-holding ability (hydrophilicity) of the concentrates, the soluble nitrogen content ($r^2 = 0.97$) and the foaming ability ($r^2 = 0.92$). Foam stability is positively correlated with emulsion capacity ($r^2 = 0.75$) and negatively correlated with pH ($r^2 = 0.86$) and foaming ability ($r^2 = 0.56$). Hydrophobes are thought to cause foam collapse by competing with protein at the bubble surface, thus disrupting the continuity of the adsorbed protein film (Hart 1986).

Colour of MBC

There is little colour difference between MBC-pI and SPC. The colour was found to be affected by pH, moisture, protein and fat content and soluble N of the concentrates.

Effect of the MBCs on the Properties of Fish Sausage

When the MBCs were added, the properties of the concentrates affected the properties of the fish sausage (*Figs. 1* and *2*). The percentage of protein in the concentrate is positively correlated with the organoleptic texture score ($r^2 = 0.75$). The increase in firmness with the addition of MBCs was preferred by the panellists over the control, while addition of SPC was disliked.

The percentage of soluble nitrogen in the protein concentrates favourably affected the sausage weight loss ($r^2 = 0.78$), shrinkage ($r^2 = 0.71$) and Instron-measured firmness ($r^2 = 0.92$). The fish sausage samples which contained protein concentrates were preferred to the normal fish sausages for flavour and texture.

Increasing the pH of the protein concentrates reduced weight loss ($r^2 = 0.50$), shrinkage ($r^2 = 0.89$) and Instron measured firmness ($r^2 = 0.85$) but decreased the flavour ($r^2 = 0.55$) and sensory scores for juiciness ($r^2 = 0.83$). This can be attributed to the increased net charge on the MBCs with increasing pH
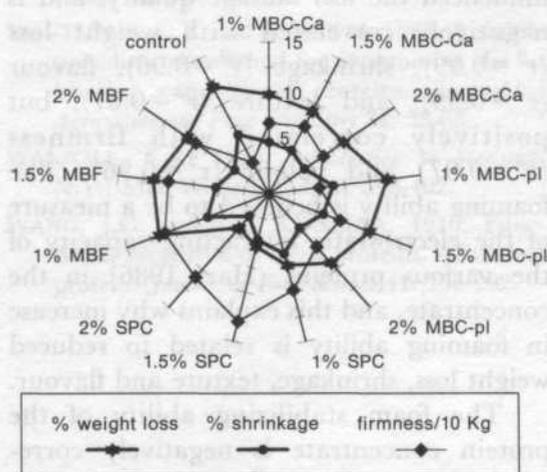

Fig. 1. Effect of protein concentrate on weight loss, shrinkage and firmness of fish sausages

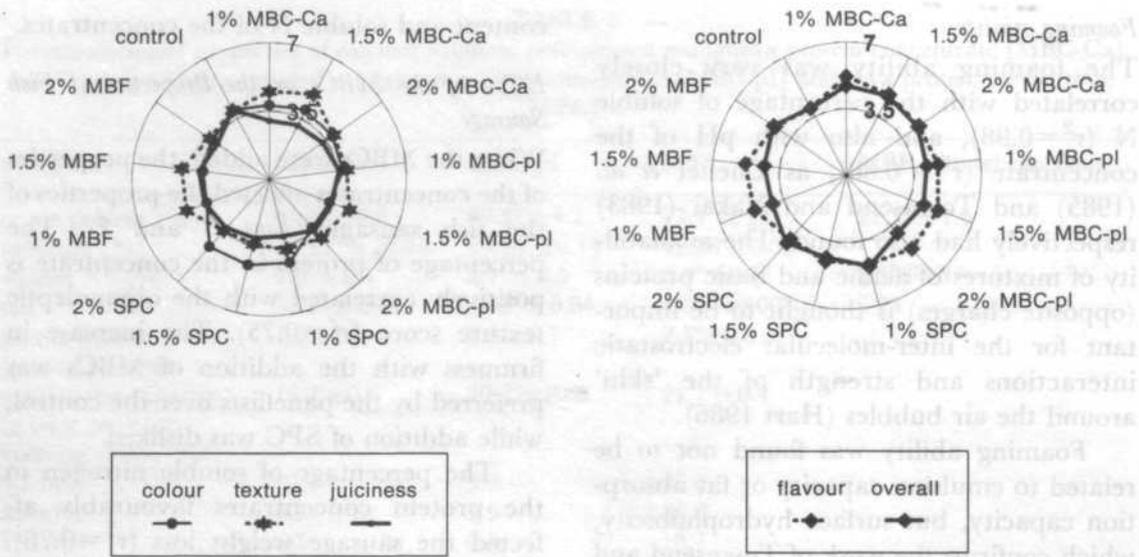colour   texture   juiciness

flavour   overall

Fig. 2. Sensory evaluation of fish sausages containing plant proteins

above the pI, thus increasing the swelling and the water-binding ability of the MBCs.

The emulsifying capacity and fat absorption capacity do not have much effect on the fish sausage. This functional property may have more influence in meat sausages, which have a greater percentage of fat included in the formulation.

The MBC foaming ability greatly influenced the fish sausage quality, and is negatively correlated with weight loss ($r^2 = 0.99$), shrinkage ($r^2 = 0.96$), flavour ($r^2 = 0.99$), and texture ($r^2 = 0.67$), but positively correlated with firmness ($r^2 = 0.97$) and colour ($r^2 = 0.96$). The foaming ability is believed to be a measure of the electrostatic interacting capacity of the various proteins (Hart 1986) in the concentrate, and this explains why increase in foaming ability is related to reduced weight loss, shrinkage, texture and flavour.

The foam stabilizing ability of the protein concentrate is negatively correlated with shrinkage ($r^2 = 0.99$) and weight loss ($r^2 = 0.56$), and has been shown earlier to be correlated with the hydrophilicity of the concentrate.

In all experiments the weight loss of fish sausage was reduced by adding protein concentrate (*Fig. 1*). The original sausage weights were better retained with SPC > MBC-Ca > MBC-pI > MBF. Weight loss is mainly due to the reduction in water-holding capacity of the heat-denatured protein. Adding the protein concentrates also increased the firmness of the fish sausages in the following order SPC > MBF > MBC-Ca > MBC-pI. Sensory evaluation (*Fig. 2*) showed that the samples of fish sausage with added plant protein were preferred (overall acceptability) to the control sausages. Generally, there was no significant difference in texture and flavour, but a decrease in juiciness was observed in the fish sausages with added plant protein. Colour scores for the sausages was higher with the addition of SPC. The colour scores for sausages with added MBC-Ca were insignificantly different from those of the control, but those with MBF or MBC-pI scored slightly unfavourably.

CONCLUSION

Incorporation of mungbean protein concentrate increased the overall acceptability of fish sausages by reducing the weight loss, shrinkage and increasing their firmness.

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