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COMMUNICATION III

Formation of Protein Film from Chickpea Dhal

ABSTRACT

The optimum conditions for the formation of chickpea dhal protein film and the characteristics of the films were studied. Optimum yield of protein films was obtained form chickpea dhal at bean: water ratios of 1:5 to 1:7 and in the pH range of 9.0 to 10.0. The chickpea protein films were darker and more brittle than soybean protein films.

ABSTRAK

Keadaan optimum pembentukan selaput protein dan ciri-ciri selaput protein yang terbentuk daripada kacang kuda telah dikaji. Hasil selaput protein yang paling baik didapati daripada dhal kacang kuda dengan nisbah kekacang dan air 1:5 hingga 1:7 dalam julat nilai pH antara 9.0 hingga 10.0.Selaput protein kecang kuda lebih gelap dan rapuh daripada selaput protein kacang soya (fucuk).

INTRODUCTION

The production of protein film from legumes not only increases legume utilisation but also results in a highly nutritious, versatile food. The film can be used in soups, vegetable or meat dishes and as an edible wrapping film. Protein films have been made from soy milk, and soy protein isolates (Watanabe *et al.,* 1975), peanut milk (Aboagye and Stanley, 1985), cotton seed milk, skimmed milk, whey proteins, caseins (Wu and Bates, 1973), keratin (Okamoto, 1978; Anker, 1972), and winged bean milk (Soleha Ishak, 1985). The film formation is believed to be an endothermic polymerisation of heat denatured protein, simultaneous with surface dehydration (Wu and Bates, 1972).

Chickpea ranks among the world's three most important pulse crops with a total annual production of round 7 million tonnes of dry seed from an area of about 10 million hectares (Summerfield & Roberts, 1985). Chickpeas have the advantage of a higher fat content than most pulses, which not only gives a higher energy concentration but imparts a smoother texture and makes very palatable products (Aykroyd, 1982). The present work attempts to study the formation of protein films from chickpea dhal.

MATERIALS AND METHODS

Dried dehulled chickpea (dhal) were obtained from the retail shops at Sri Serdang. The beans were hand sorted to remove foreign material, weighed, washed and soaked in excess water overnight. The beans were rinsed and blended in water for about 3 minutes. The amount of water used for extraction was varied to determine the optimum bean-to-water ratio to provide the maximum film yield. The slurry obtained was converted to a milk by filtration using an extractor and a hydraulic press.

To study the best pH for optimum film formation, the milk was adjusted to the desired pH value (using 1M NaOH), within the range of pH of 8.0 to 11.0, since no chickpea protein film could form below pH 8.0.

The protein film was formed by the irreversible insolubilisation of dhal milk during evaporation at 85°C from 600 ml milk in a shallow tray (24 cm x 14 cm x 5 cm). Films were harvested at 20 minutes intervals by loosening the film from the edges of the tray and picking it up with a slender glass rod. Film formation was continued until film could no longer form. At this moment 100 ml of distilled water was added to the milk to reduce its viscosity and film formation was continued. The films were hung in air to dry at room temperature for 24 hours and the colour and weight of each film was recorded.

Colour was determined using the Hunter Tristimulus Colorimeter for L, a, and b values (lightness, redness and yellowness) using the yellow tile (L= 77.5, a= 3.5, b= 23.0) as standard. The protein content of each film was determined using the micro Kjeldahl method (Pearson, 1976). Values presented are the average readings of 6 different trials.

RESULTS AND DISCUSSION

Chickpea dhal protein film was darker in colour and more brittle than soybean protein film. The darker colour could be due to the high lysine and carbohydrate content of chickpea which provides conditions favourable for non-enzymic browning (Maillard reaction) at the high temperature and alkaline conditions for film formation.

The colour of chickpea protein became darker as the pH of the milk was increased above the neutral pH *(Figs. 1, 2 and 3)*, indicated by the decrease in L (Lightness) and b (Yellowness) values.

Although alkaline pH favours more complete protein extraction, it promotes the Maillard reaction between the free amino groups with the carbohydrate present, to form brown nitrogenous polymers and copolymers (melanoidins) (Lee, 1977). The surface of the film became less wrinkled and smoother with increasing pH. Similar observations were reported by Aboagye and Stanley (1985) on peanut protein lipid films. This is attributed to the enhanced protein unfolding under alkaline conditions and the changed surface charge distribution which affected the relative attractions of proteins to the liquid surface, manifested by a decrease in surface tension. The films formed at high pH tended to be staighter with less intramoleculer elasticity. At alkaline pH the extracted milk was more viscus, indicating that the protein molecules in solution were more linear and less globular. Alkaline conditions may also cause protein peptidization and thus may effect alkali labile amino acids which decrease the biological value of the protein. Thus, the general characteristic of the protein film was more favourable at higher pH, even though the product was darker in colour.

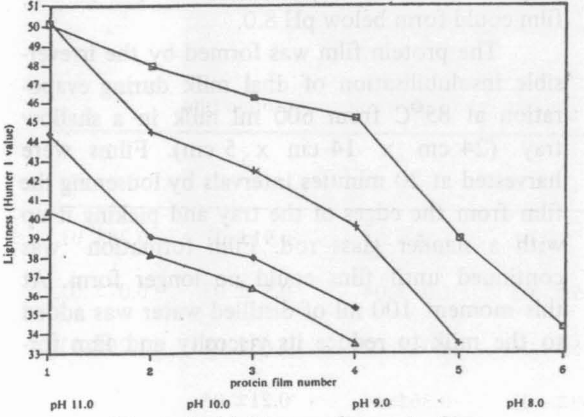

Figure 1: Lightness of protein film.

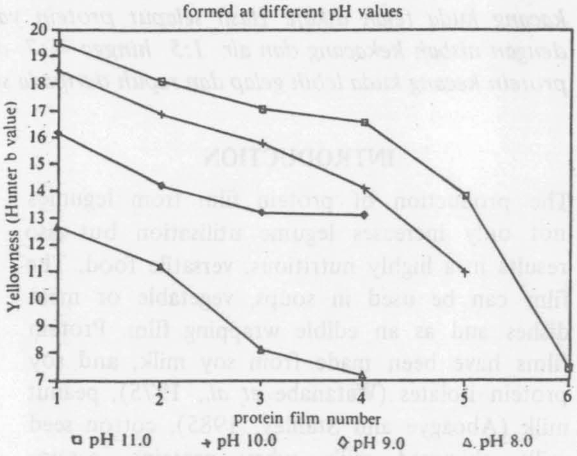

Figure 2: Yellowness of protein film made

The film brittleness may be explained by the low protein and cysteine of chickpea compared to soybean. Hence the formation of less intermolecular disulphide bonds and the lower breaking point (yield stress). The brittleness could also partly be due to the interference of a strong protein-protein interaction by the higher amount of carbohydrate that may have been incorporated into the film, since chickpea has a much higher carbohydrate content than soybean.

Wu and Bates (1973) reported that films formed from soya protein isolate (SPI) in the presence of added lipid or carbohydrate are smoother and of a more even texture than those formed from SPI alone, which tended to from cracked films. Their studies indicated that the presence of secondary components is essential for good films formation of improved quality, but that formed at different pH values

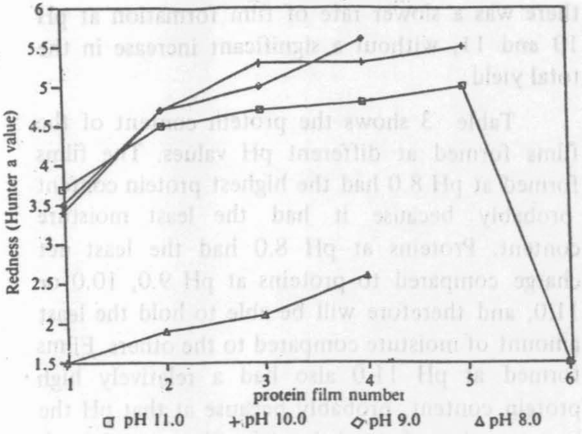

Figure 3: Redness of protein film

excessive amounts of such components were detrimental.

Work by Okamoto (1978) showed that the soybean protein films had greater tensile strength when the films were formed on a water permeable solid/liquid interface rather than a gas/liquid interface. The water permeable solids which were successfully tested were cellophane and acetyl cellulose. The same method can probably be applied to improve and increase the tensile strength of chickpea protein films. The formation of chickpea protein films in the presence of solubilised wheat gluten or keratin might also increase the strength of chickpea protein films, since gluten and keratin formed very strong pliable films on their own (Okamoto, 1978).

The protein film yield was maximum when the bean: water ratio was between 1:5 and 1:7 (Table 1). *Fig. 4* shows the protein content of films made with different bean to water ratios, indicating a reduced protein content when the bean to water ratio was decreased below 1:7. Bean to water ratios below 1:5 resulted in incomplete protein extraction from the dhal, while too great a dilution hindered film formation. Data on the protein yield in each film (Table 1) shows that there is an increase in protein yield in the second film with decreasing protein yield thereafter.

The protein film could form only in alkaline pH, and the optimum yield (g of film) was obtained at pH 9.0 (Table 2), indicating the important role of electrostatic repulsion for chickpea protein denaturation. Between pH 8 and pH 9, most of the alfa amino groups begin to lose their positive charges hence causing a slight change in the net negative charge in the protein molecules. The intramolecular repulsion and high temperature favours unfolding of the protein molecules and exposure of the hydrophobic side chains. In aqueous solutions, the hydrophobic side chains would either interact with each other to form hydrophobic bonds or be pushed to the air/water interface. This phenomena together with the evaporation of water at the surface, helped produce a high protein concentration at the air/water interface which promotes intermolecular interaction of the unfolded protein, and this results in film formation. Above pH 9.5, other charged side chains such as the epsilon group of lysine, the hydroxyl group of tyrosine and the SH group of cysteine began to lose their charges causing a drastic change in the net negative charge on the protein molecule, which not only resulted in intramolecular repulsion but also intermolecular repulsion, thus hindering film formation. However the relative rates of these reactions not only depend on the severity of the

TABLE 1

Effect of bean: water ratio on the protein content (g) of each chickpea protein film

Film no. / b:w	1	2	3	4	5	6
1 : 5	1.77±.01	2.28±.02	1.7 ±0.01	1.21±.01	0.91±.01	0.38±.01
1 : 6	1.77±.02	2.27±.05	1.25± .03	1.0 ±.04	0.5 ±.03	0.07±.01
1 : 7	1.55±.01	1.82±.04	1.16± .04	1.0 ±.02	1.63±.01	1.20±.01
1 : 8	0.71±.01	0.99±.01	0.49± .01	0.36±.01	0.21±.01	

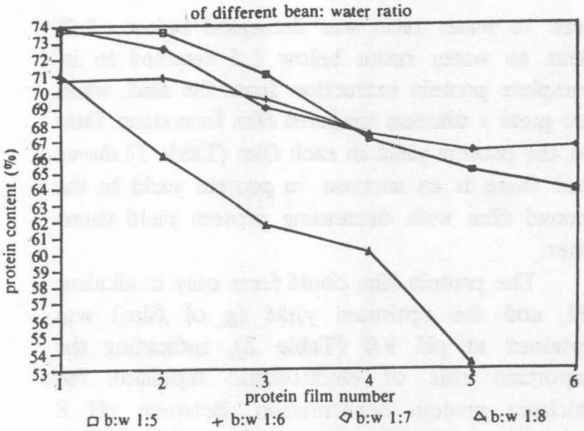

Figure 4: Protein content in chickpea films

conditions but also on the type of protein under treatment, and results which are severe for one protein may not be for another. For chickpea, there was a slower rate of film formation at pH 10 and 11, without a significant increase in the total yield.

Table 3 shows the protein content of the films formed at different pH values. The films formed at pH 8.0 had the highest protein content probably because it had the least moisture content. Proteins at pH 8.0 had the least net charge compared to proteins at pH 9.0, 10.0 or 11.0, and therefore will be able to hold the least amount of moisture compared to the others. Films formed at pH 11.0 also had a relatively high protein content, probably because at that pH the incorporation of starch into the film was minimal. Although protein films consists mainly of protein membranes, lipids and carbohydrate incorporated

TABLE 2

Yield of protein film (g) at different pH

pH	Time of harvest (mins)						Total wt. g	% yield g/100 g beans
	20	40	60	80	100	120		
11.0	1.9 (1.9)	2.1 (4.0)	2.1 (6.1)	1.6 (7.7)	0.6 (8.3)	0.2 (8.5)	8.5	10.0
10.0	1.9 (1.9)	2.4 (4.3)	1.7 (6.0)	1.2 (7.2)	0.9 (8.0)		8.1	9.5
9.0	2.5 (2.5)	2.3 (4.8)	1.9 (6.7)	1.3 (8.0)			8.0	9.4
8.0	1.8 (1.8)	1.8 (3.6)	1.1 (4.7)	0.8 (5.5)			5.5	6.4

(Values in brackets are the total yield at any given time).

TABLE 3

Effect of pH on the protein content (g) of each chickpea protein film

pH	Film No.					
	1	2	3	4	5	6
8	72.2 (1.3)	71.9 (2.59)	71.7 (3.38)	69.3 (3.93)		
9	68.3 (1.71)	67.2 (3.26)	66.7 (4.53)	60.9 (5.32)		
10	68.9 (1.31)	67.9 (2.94)	67.0 (4.08)	66.5 (4.88)	61 (5.43)	
11	70.7 (1.34)	71.6 (2.84)	68.9 (4.29)	66.1 (5.35)	66.1 (5.75)	63.6 (5.88)

(Values in brackets are the total protein yield at any given time).

TABLE 4
Composition of chickpea compared to soybean (g/100 g seed) (Aykroyd, 1982)

Legume	H_2O	Protein	Fat	Sugar	Starch	CHO*	Fibre	M	C	T	W	V
Chickpea	9.9	20.6***	5.6	10.0	40.0	50.0	15.0	80	90	240	50	240
Soyabean	7.0	36.8**	23.5	11.2	12.3	23.5	11.9	80	100	240	80	30

CHO* = Total carbohydrate; ** N x 5.71 *** N x 6.25;
Fibre = Total Fibre; M = methionine; C = cysteine;
T = threonine, W = tryptophan; V = valine;

into it during film formation contribute significantly to the flavour and physical properties of the film (Watanabe *et al.,* 1975).

The total weight of protein film which can be obtained from 100 g soybean was between 22 g to 31 g. This is about 2 to 3 times higher than that obtained from the same weight of chickpea (Table 2). The reason for the lower yield of protein film from chickpea is the lower protein content of chickpea (about 20%) compared to protein content of soybean (about 40%) and the possible interference of protein film formation by the high carbohydrate content in chickpea (Table 4).

Further work on determination of the characteristics of the chickpea protein film need to be done before its commercial potential can be assessed. The characteristics include sensory evaluation (acceptability); permeability to moisture or oxygen for use as edible barrier; and the possibility of forming other shapes eg. cylinders (for sausage casings) using cellophane or acetyl cellulose as the water permeable solid/liquid interface mould, while helping to increase the tensile strength of the film.

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