

EFFECT OF PRESSURE LEVEL AND SLURRY PARTICLE SIZE ON SOLIDS AND PROTEIN EXTRACTABILITY DURING COLD EXTRACTION

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Abstract

Aim of this study was to obtain information on the effects of extraction pressure and slurry particle size on the level of solids and protein extractability in soymilk.

A completely randomised factorial design was employed in this series of the experiments. The experiments were carried out in duplicate. Two factors were varied : pressure level (2.5; 5; 10; 15; and 20 psi) and particle size of the slurry.(coarse, medium, fine, and very fine slurry).

Results showed that both the level of pressure and the slurry particle size played a key role in solid and protein extraction. Increasing the pressure while reducing the slurry particle size resulted in raising the yield and total solid, but not the protein content.

The solid and protein extractability increased by applying a higher pressure on the finer slurry particle size. The highest extractability of solid and protein was 67.61 and 83.46%, respectively, achieved by the very fine slurry pressed at 20 psi. Whereas the coarse slurry produced the lowest result namely 57.69% for solid extractability and 72.27% for protein extractability.

INTRODUCTION

Tofu (soybean curd) is one of the most important non-fermented food products used widely in East Asia and South East Asia. In the United States and Canada, production of tofu is increasing due to an increase of Asian immigrants and acceptance of the product by the general population (Lim *et al.*, 1990^a). There is a large growing interest in Oriental food, especially tofu, in the American market outside the Oriental community (Wang, 1984). In other words, this traditional food is rapidly gaining popularity in the West (Mohamed *et al.*, 1989).

Tofu is made by coagulating soybean protein with salt or acid from a water extract of whole soybeans. It is a highly digestible and nutritious food which represents an inexpensive source of protein (Kamel and deMan, 1982; Shurtleff and Aoyagi, 1983). Moreover, it contains fewer calories and saturated lipids than other protein-rich foods, is a good source of linoleic acid (an essential fatty acid) and contains no cholesterol (Mohamed *et al.*, 1989).

For centuries, the process of making tofu has been controlled by tradition and long experience (Wang *et al.*, 1983). Today, tofu is prepared in essentially the same way that it was more than 2,000 years ago (Wang and Hesseltine, 1982). Basically, the production of tofu consists of two main steps (Shurtleff and Aoyagi, 1983) : (1) preparation of soymilk; and (2) the coagulation of this soymilk to form curd which is then pressed to form tofu cakes.

In recent years, various research projects have analysed the processing conditions in an effort to improve the yield and quality of tofu. Most of those projects have concentrated on the raw material or soybean variety and various coagulation treatments.

The use of different varieties of soybeans and varying treatments of the soybean before processing has been employed by Wang *et al.* (1983), deMan *et al.* (1987), Lim *et al.* (1990^b), and Metussin *et al.* (1992). Research examining both the raw material and coagulation treatments has been done by Watanabe *et al.* (1964), deMan *et al.* (1975), Lu *et al.* (1980), Tsai *et al.* (1981), Kamel and

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deMan (1982), Beddows and Wong (1987^{b,c}), Sun and Breene (1991), and Shen *et al.* (1991).

Surprisingly, very little research has been undertaken on the preparation of soymilk especially, the extraction process. Some work in this area has been done by Watanabe *et al.* (1964), and Beddows and Wong (1987^a) investigated the effects of water:beans ratio in the extraction process.

The amount of extracted solids and proteins in the milk greatly determines the yield and the properties of tofu. Total solids content of soymilk is the only parameter that shows a positively significant correlation with fresh yield of tofu (Lim *et al.*, 1990^b), so that estimation of solids extractability from soybeans could be used as a quality control tool in tofu production (Shen *et al.*, 1991). In addition, soymilk with high protein content will produce tofu with high protein content as well (Lim *et al.*, 1990^b).

Although various studies have investigated the process of the production of tofu, the effects of solids and protein extraction still need further experimentation. In addition, a study which examines the extraction process (especially solids and protein extraction) in detail, should yield information about the nature of the extraction process. There is very little detailed information in this important area.

The aim of this study was to obtain information on the effects of extraction pressure and slurry particle size on the level of solids and protein extractability in soymilk.

METHODOLOGY

A. Experimental Design

A completely randomised factorial design was employed in this series of the experiments. The experiments were carried out in duplicate. Two factors were varied : pressure level and particle size of the slurry. Levels of each factor are given below :

Factor I : Pressure level (P)

P1 : 2.5 psi (0.176 kg/cm²); P2 : 5 psi (0.352 kg/cm²); P3 : 10 psi (0.703 kg/cm²); P4 : 15 psi (1.055 kg/cm²); and P5 : 20 psi (1.406 kg/cm²).

Factor II : Slurry particle size (S)

S1 : 76.91% particles passed through a 250µm aperture (coarse slurry);

S2 : 83.23% particles passed through a 250µm aperture (medium slurry);

S3 : 86.05% particles passed through a 250µm aperture (fine slurry); and

S4 : 88.22% particles passed through a 250µm aperture (very fine slurry).

Analysis of the soymilk included yield of milk, total solids and protein content.

B. Soymilk preparation

Four grinding treatments were used to produce four different slurry particle sizes (Yuwono, 1996) and five treatments of pressure level were for expression treatments. The resultant slurries were finally filtered through a layer of 250-315 µm aperture size cotton cloth. A flow diagram for the experiments is shown in Figure 1.

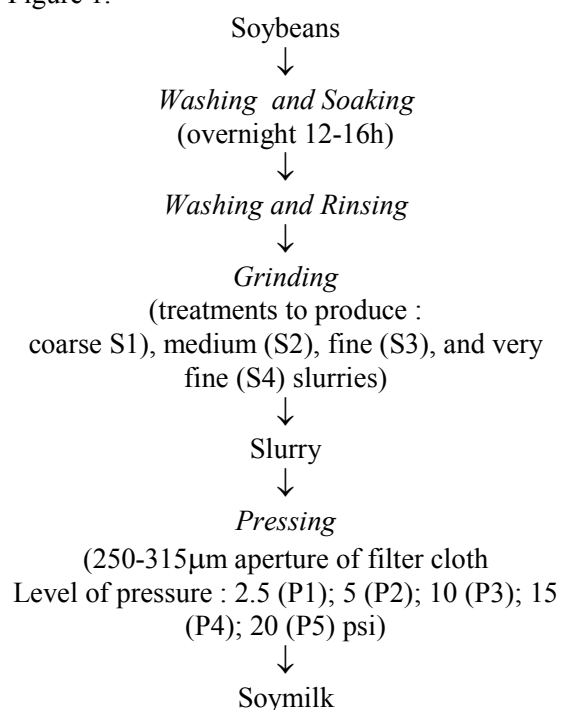


Figure 1. Flow diagram of Experiment

RESULTS AND DISCUSSION

A. Physical and chemical properties of the soybeans

Soybeans used as raw material were in the size range 17.15 ± 0.20 g/100 seeds (dry basis). This size range is similar in range to the size of beans (15.24 to 35.51 g/100 beans) used by Wang *et al.* (1983) or 8.71 to 41.27 g/100 beans used by Lim *et al.* (1990), however is smaller than the size of 17.2 to 23.3 g/100 beans

higher yield of soymilk. Because of the interaction effect between the two factors, a least significant difference analysis was carried out on the interaction, in order to determine the differences between the levels. Results are summarised in Table 1.

This experiment confirmed that the

Table 1. Effect of slurry particle size and pressure level on means of yield of milk (%)*.

Slurry particle size	Level of pressure (psi)				
	2.5	5.0	10.0	15.0	20.0
88.22% pass 250 μ m	82.31 bc	84.19 de	85.14 ef	86.05 fg	86.68 g
86.05% pass 250 μ m	82.07 b	83.87 d	84.70 e	85.54 f	86.05 fg
83.23% pass 250 μ m	81.67 ab	83.38 cd	84.26 de	84.75 e	85.22 ef
76.91% pass 250 μ m	81.19 a	81.90 b	82.47 bc	82.73 c	82.97 c
LSD** : 0.69					

*) Means followed by the same letter are not significantly different ($P < 0.05$)

**) Least Significant Difference

used by deMan *et al.* (1987). It is debatable whether the size range of the soybeans has any effect on the properties of the soymilk or tofu (Lim *et al.*, 1990) although it was kept constant throughout this experimentation in an attempt to avoid any unwanted variables.

The moisture content and protein of the beans were $8.5 \pm 0.49\%$, and $33.76 \pm 0.34\%$ (dry basis) respectively. The protein content of the beans used for these experiments was in the range of protein content (32.4 to 38.2%) used by Skurray *et al.* (1980), much lower than American or Japanese soybeans used by Wang *et al.* (1983) which ranged from 40.8 to 45.2%, or the Canadian soybeans used by deMan *et al.* (1987) which ranged from 38.1 to 43.1%.

B. Soymilk and its properties

a. Yield of milk

The processes of pressing and grinding prior to cold extraction, are necessary for a high yield of soymilk. The yields ranged from 81.05 to 86.71%. A significant effect on the yield was shown by both level of pressure and particle size of the ground soybeans and an interaction between the factors also occurred. An increase of pressure level in conjunction with a reduction in slurry particle size has produced a

finer the slurry and the higher the pressure level, the more yield to be obtained. A pressure of 20 psi and 88.22% particles of the slurry particles passing through a 250 μ m aperture (very fine slurry) gave the highest percentage of yield, whereas 2.5 psi level of pressure and 76.91% particles passing through a 250 μ m aperture size sieve (coarse slurry) produced the lowest yield.

The effects of the level of pressure during expression and slurry particle size at that pressure is shown graphically in Figure 2. Analysis has shown that a pressure increase from 2.5 to 5 psi produces a significant rise in yield for all slurry sizes. After 5 psi, the increase in yield is affected by the slurry particle size. However, in all cases, after the pressure reaches 15 psi, there is no significant effect on the yield as the pressure is increased to 20 psi.

The fine particle size slurries appear to respond to the effects of pressure more than the coarse particle size slurries when yield is considered (Figure 2). Increasing the pressure considerably improved the yield with the fine slurry, due to an increase in the extractable substances. From Table 1, it can be seen that

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increasing the pressure had a similar effect on the yield in fine and very fine slurries.

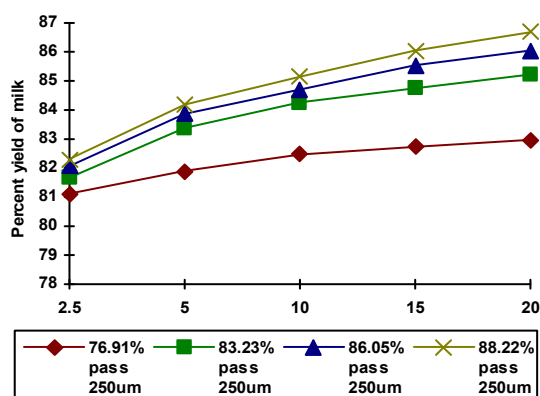


Figure 2. Effect of level of pressure and particle size of the slurry on yield of milk

The curve for the medium slurry is different to that of the fine and very fine slurries. For the medium slurry, increasing the pressure produced a significant increase in yield until the pressure exceeded 10 psi. Increasing the pressure from 10 to 15 psi had no significant effect on the yield. However, at 20 psi the yield is significantly different to that at 10 psi, but not 15 psi.

A slight increase in yield as an effect of increasing the pressure was shown by the coarse slurry. Table 1 indicates that 5 psi is the critical pressure for the coarse slurry as there was a significant increase in yield when compared to the yield at a pressure of 2.5 psi. After this point (pressure at 5 psi), increasing the pressure above 5 psi, had no significant effect on yield. These results indicate that for the coarse slurry, increasing the pressure above 5 psi level had no significant effect on the yield, possibly due to the large particle size of the ground soybeans.

b. Total solids

Total solids is one of the most important parameters in soymilk. A higher total solids in the soymilk resulting from the same water:bean ratio indicates a more efficient extraction. Statistical analysis of the results has

shown that the level of pressure and the particle size of the slurries resulted in a significant effect on the total solids, however, no significant interaction occurred between the factors. Total solids ranged from 5.91 to 6.48%. In general, even though no significant interaction occurred between the factors, a trend can be observed. As the level of pressure increase and the slurry particle size decrease, the total solids tend to increase (Figure 3).

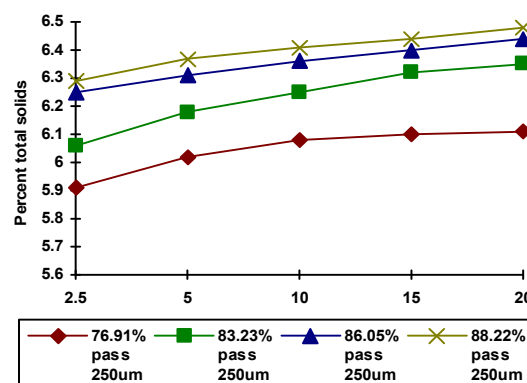


Figure 3. Effect of pressure level and slurry particle size on total solids

From this figure, it can be seen that each slurry responds differently to the increase in pressure. Coarse and medium slurries produce a marked increase in total solids from 2.5 to 5 psi; and 2.5 to 15 psi respectively. After this point, an increase in the pressure level only resulted in a slight increase in the total solids. In other words, pressure at 10 and 15 psi has provided for a useful increase in total solids for the coarse and medium slurries, respectively. However, increasing the extraction pressure of fine and very fine slurries steadily increased the total solids of the soymilk produced.

Figure 3 shows that increasing the pressure has produced only a slight increase in total solids in either the coarse, fine or very fine slurry. Conversely, the greatest increase in total solids as affected by pressure is shown by the medium slurry.

From these trends, it can be shown that the re-grind process (fine and very fine slurries) greatly reduced the particle size, and increased

the extraction of solids. Hence, it could be assumed that for both fine and very fine slurries, most of the soluble solids have already been extracted before the expression process. The expression process for both the fine and very fine slurries may be utilised to separate more extracted solids (produced by grinding) from the okaras. Therefore, at the low pressure level, the total solids of soymilk from both the fine and very fine slurries was higher than that of soymilk from either the medium or coarse slurries produced at the high pressure. As a result, there was only a slight increase in total solids as an effect of increasing the pressure from 2.5 to 20 psi for the fine or very fine slurries (an increase of 0.15% for very fine slurry and 0.17% for fine slurry).

With coarse or medium slurries, increasing the pressure is used to extract the solids which have already been extracted by the grinding action and to press out soluble solids inside the okaras. At low pressure (2.5 psi) the milks contained a low percentage of total solids (6.06 and 5.91% in milk from medium and coarse slurries, respectively), possibly due to the pressure being not high enough to press out the soluble solids from the ground cotyledons. However there were differences between the coarse and medium slurries. The coarse slurry had a lower increase in the total solids of the soymilk when compared to the medium slurry, due to the larger size of okaras. The total solids in the soymilk from the coarse slurry increased by 0.2% and 0.29% in milk from the medium slurry by increasing the pressure from 2.5 to 20 psi. Effect of slurry particle size on total solids is shown in Table 2.

Table 2. Effect of slurry particle size on total solids.*

Particle size of the slurries	Total solids (%)
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76.91% pass through 250µm	6.044 a
83.23% pass through 250µm	6.230 b
86.44% pass through 250µm	6.354 c
88.22% pass through 250µm	6.402 d
LSD **	0.032

*) Means followed by the same letter are not significantly different ($P < 0.05$)

**) Least Significant Difference

A consistent increase in the total solids by reducing the particle size of the ground soybeans is shown in Table 2. Similar to the early results discussed which indicated that the finer the slurry, the higher total solids, it can be seen that as the slurry particle decreases, the total solids increases. This could be due to the greater disruptive action of grinding which could displace more soluble solids from the cell and produce more okaras sized less than 250 µm. The very fine slurry (88.22% particles passed through a 250 µm aperture) produced the highest total solids, whereas, the coarse slurry (76.91% particles passed through a 250 µm aperture) had the lowest solids.

When considering the level of pressure, total solids are affected by the pressure applied in expression. Generally, the higher the pressure, the greater the yield of solids. Effect of pressure level on total solids is shown in Table 3.

Table 3. Effect of pressure level on total solids.*

Level of Pressure (Psi)	Total Solids (%)
2.5	6.140 a
5.0	6.218 b
10.0	6.273 c
15.0	6.314 d
20.0	6.344 d
LSD **	0.036

*) Means followed by the same letter are not significantly different ($P < 0.05$)

**) Least Significant Difference

When comparing the different pressure levels, a significant difference in total solids was affected by pressures of 2.5, 5, 10, and 15 psi. However, neither 15 nor 20 psi gave any

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significant difference in total solids. This may be due to after pressing at 15 psi the soluble solids content in ground soybeans (okaras) is relatively low, so that pressing out remaining soluble solids needs a pressure significantly higher than 20 psi.

c. Protein content

One of the aims of the extraction process, is to extract as much protein as possible. This experiment showed that level of pressure had no significant effect on the protein content of the soymilk. However, similar to the earlier experiments, slurry particle size did have a significant effect on protein content, and a significant interaction between the factors occurred. Effect of pressure level and slurry particle size on protein content (dry basis) is shown in Table 4.

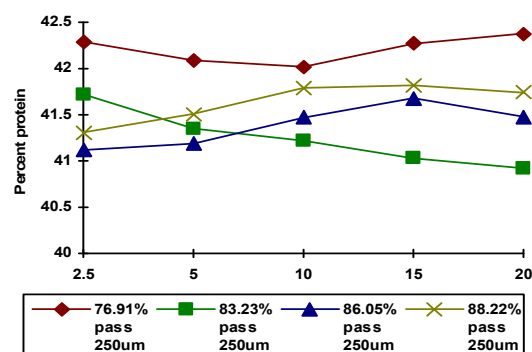


Figure 4. Effect of pressure level and slurry particle size on protein content of soymilk

A similar trend on protein content was produced by coarse and medium slurries when they were pressed at 2.5 to 10 psi. The protein content decreased by increasing the pressure.

Table 4. Effect of pressure level and slurry particle size on protein content (dry basis)*

Slurry particle size	Level of pressure (Psi)				
	2.5	5.0	10.0	15.0	20.0
88.22% pass 250µm	41.13 ab	41.15 ab	41.79 bc	41.82 bc	41.74 bc
86.05% pass 250µm	41.12 ab	41.19 ab	41.47 b	41.68 bc	41.48 b
83.23% pass 250µm	41.72 bc	41.35 ab	41.22 ab	41.03 ab	40.92 a
76.91% pass 250µm	42.29 c	42.09 c	42.02 c	42.27 cd	42.38 cd
LSD** : 0.52					

*) Means followed by the same letter are not significantly different ($P < 0.05$)

**) Least Significant Difference

The protein content of the soymilk ranged from 40.71 to 42.39% on a dry basis. In all pressure treatments, the coarse slurry still produced the highest protein content, even though no significant difference occurred between the pressure levels. Fine and very fine slurries responded in a similar way to increasing pressure levels. However, medium slurries produced contradictory results showing a decline in protein content as the pressure level was increased. In order to examine any possible trends in level of protein as affected by extraction pressure levels, the data from Table 4 have been presented graphically in Figure 4.

However after 10 psi of pressure, an increase of pressure produces a slight increase in protein content of soymilk from the coarse slurry, but not for the medium slurry. This result may have been caused by the relatively smaller particle size in the medium slurry when compared to the coarse slurry resulting in an increase in the degree of extraction non-nitrogenous soluble substances other than protein. In other words, a higher proportion of non-nitrogenous compounds are extracted than nitrogen compounds (protein).

A similar trend also is shown by the fine and very fine slurries, which showed an increase in protein content by pressing from 2.5

to 10 psi. An increase in the protein content of milk from the fine slurry still continued when the pressure extended to 15 psi. Conversely, the protein content of milk from the very fine slurry decreased by increasing the pressure above 10 psi. Because of the double extraction (re-grind) process, most of the proteins may be already extracted in the grinding process for the fine and very fine slurries. Hence increasing the pressure with these slurries could be used to separate the extracted proteins from the okaras resulting in an increase of protein content. However, when a high level of pressure was applied, protein extraction was also followed by non-nitrogenous soluble solid extraction producing a decrease in the overall protein content.

C. Extractability of solids and protein

a. Solids extractability

The amount of extracting solids and proteins is very important in the extraction process. As was done previously, extractability is presented by either SSR (soymilk solid recovery) or SPR (soymilk protein recovery). From earlier experiments, it was shown that the particle size of ground soybeans had a significant effect on the extractability of solids and protein. In this experiment, slurry particle size and pressure level was shown to have an important role in extraction. A significant difference was resulted from the particle size of ground soybeans and level of pressure, and that significant interaction between the factors occurred in SSR. The results of the effect of slurry particle sizes and level of pressure on SSR are summarised in Table 5.

Extractability of solids ranged from 57.87 to 84.17%. Raising the pressure followed by reducing the slurry particle size caused an increase of solids extractability. However, not all increases of pressures produced a significant difference in solids extractability. Increasing the pressure above 15 psi had no significant effect on solid extractability for the medium slurry. The same figure occurred after 10 psi of pressure for the coarse slurry. Table 5 shows that increasing the pressure produced a significant difference in extractable solids from

both the fine and very fine slurries. However, for a clearer understanding of the effect of slurry particle size and level of pressure could have on solids extractability, data from Table 5 have been presented graphically in Figure 5..

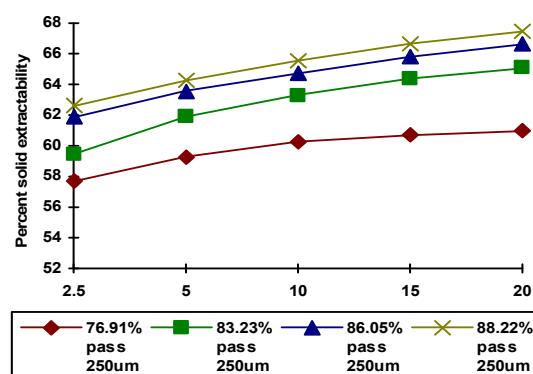


Figure 5. Effect of pressure level and slurry particle size on solid extractability recovered in soymilk (SSR).

Figure 5 portrays the effect of level of pressure and slurry particle size have on solid extractability. It can be seen that in the medium slurry, an increase in the solids extractability is achieved as the level of pressure is increased. An increase of pressure from 2.5 to 20 psi resulted in a rise of 5.6% solid extractability. Whereas, fine and very fine slurries, at the same range of pressure gave an increase of only 4.87 and 4.75%, respectively. Coarse slurry produced the lowest increase at 3.23%.

From this data, it can be concluded that, the extractability of solids from the medium slurry greatly depends on the pressure level. Whereas, in the case of fine and very fine slurries, the solid extractability depended less on the pressure level and the solid extractability for the coarse slurry was only slightly affected by the pressure level.

These findings strengthen the previous conclusions that pressure applied to fine and very fine slurries tended to separate extracted solids already produced by the grinding action. The added pressure removes the soluble material from the very fine okaras without any further extraction of material from the okaras. Due to a relatively large okara size in the coarse

Table 5. Effect of pressure level and slurry particle size on solids extractability recovered in soymilk (SPR)
Effect of Pressure Level and Slurry Particle Size

Slurry particle size	Level of pressure (Psi)				
	2.5	5.0	10.0	15.0	20.0
88.22% pass 250 μ m	62.59 d	64.25 f	65.56 g	66.65 hi	67.45 i
86.05% pass 250 μ m	61.86 d	63.59 e	64.71 f	65.81 gh	66.61 h
83.23% pass 250 μ m	59.46 bc	61.90 d	63.31 e	64.39 f	65.06 fg
76.91% pass 250 μ m	57.69 a	59.28 b	60.26 c	60.68 c	60.95 c
LSD** : 0.81					

*) Means followed by the same letter are not significantly different (P<0.05)

**) Least Significant Difference

slurry, a slight increase of solid extractability occur by increasing the pressure.

b. Protein extractability

The amount of extracted protein ranged from 72.35 to 83.34%. Generally, the higher the pressure and the finer the slurry particles, the more protein can be obtained. A statistical analysis showed that both the slurry particle size and the pressure level had a significant effect on soymilk protein recovery, and that a significant interaction between the factors also occurred.

Increasing the pressure from 2.5 to 10 psi produced a significant effect on protein extractability in all slurries. Increasing the pressure after this level, did not cause a

(a significant difference occurs at 10 and 20 psi).

The same effect was demonstrated in the fine and very fine slurries when the pressure reached 15 psi which indicated that increasing the pressure above this figure did not result in a significant increase in protein recovery. This result may be due to a relatively low amount of extractable protein remaining in the okara after the slurry was pressed at 15 psi. Increasing the pressure by a further 5 psi (pressure at 20 psi) may not large enough to press out the remaining protein in the okaras.

In order to comprehend this trend, data from Table 6 are presented graphically in Figure 6. With regard to protein extractability, each slurry responded differently to increased

Table 6. Effect of pressure level and slurry particle size on means of protein extractability (%) recovered in soymilk (SPR)*

Slurry particle size	Level of pressure (Psi)				
	2.5	5.0	10.0	15.0	20.0
88.22% pass 250 μ m	75.77 cd	79.06 ef	80.65 g	82.55 hi	83.46 i
86.05% pass 250 μ m	75.11 c	77.58 de	79.48 f	80.96 gh	81.46 h
83.23% pass 250 μ m	73.44 b	75.32 c	77.30 de	78.25 e	78.85 ef
76.91% pass 250 μ m	72.27 a	73.91 b	75.05 c	75.97 cd	76.51 d
LSD** : 1.14					

*) Means followed by the same letter are not significantly different (P<0.05)

**) Least Significant Difference

significant difference for the coarse and the medium slurries. Because of the relatively large okara sizes, an increase in pressure of more than 10 psi may be needed to produce a significant effect with the coarse slurry. In contrast, an increase of 10 psi was enough to have a significant effect on the medium slurry

pressure. Figure 6 shows a slight difference when it is compared to solid extractability (Figure 5).

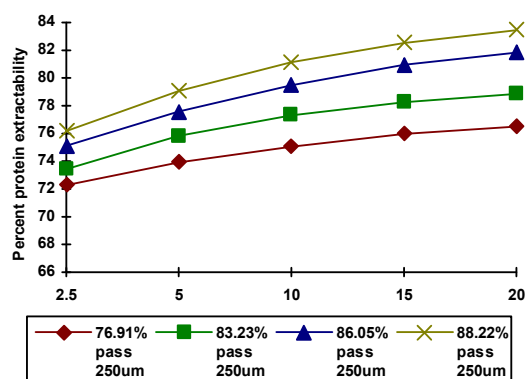


Figure 6. Effect of pressure level and slurry particle size on protein extractability recovered in soymilk (SPR).

In solid extractability the highest response resulted from the medium slurry. Whereas in the case of protein extractability the finer the slurry the greater the increase in extractability. Table 6 shows that by subtracting the extractability of protein at 20 psi with 2.5 psi, a very fine slurry produced the highest increase in protein extractability namely 7.69%. Whereas, a fine or medium slurry produced an increase of 6.35 and 5.41%. The lowest increase resulted from the coarse slurry, at 4.24%.

CONCLUSION

Both the level of pressure and the slurry particle size played a key role in solid and protein extraction. Increasing the pressure while reducing the slurry particle size resulted in raising the yield and total solid, but not the protein content.

The solid and protein extractability increased by applying a higher pressure on the finer slurry particle size. The highest extractability of solid and protein was 67.61 and 83.46%, respectively, achieved by the very fine slurry (88.22% particles passed through a 250 μ m aperture) pressed at 20 psi. Whereas the coarse slurry (76.91% particles passed through a 250 μ m aperture) produced the lowest result namely 57.69% for solid

extractability and 72.27% for protein extractability.

Each slurry responded with a different level of extracted solids as affected by an increase in the pressure. The highest response in solid extractability as an effect of pressure level was produced by the medium slurry (slurry in which 83.23% particles passed through a 250 μ m aperture). In other words, increasing the extractability of solid in the medium slurry greatly depended on the pressure level. Whereas, the coarse slurry produced the lowest response in increasing solid extractability by raising the pressure. Conversely, measuring the protein extractability from each slurry indicated that the finer the slurry the higher the increase achieved by increasing the pressure.

On the basis of these results, pressure levels at 10 and 20 psi were chosen for the next series of experiments, to represent low and high pressure levels. It was considered that 10 psi produced a reasonable solid and protein extractability, and all parameters would be significantly different from a 20 psi pressure level.

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