

Effect of coagulant type and concentration on the yield and quality of soy-lupin tofu

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Abstract

Soy-lupin tofu samples were prepared by replacing 30% soybean with lupin flour. Four different coagulants i.e. calcium sulphate, calcium lactate, magnesium sulphate and magnesium chloride were used at three different concentrations (0.3, 0.4 and 0.5% w/v of the “milk”) to study their effect on yield and quality improvement. The results revealed that the tofu samples prepared using magnesium sulphate had higher moisture content and fresh yield than those prepared from other coagulants. The L*, a* and b* colour coordinates showed no significant differences among the samples. Fat content was affected by the type and concentration of the coagulants. Magnesium sulphate and magnesium chloride at 0.5% level produced tofu with lower fat contents. Protein contents, however, were not affected by type or concentration of coagulant. Texture profile analysis revealed that the hardness and chewiness of samples changed with the type and concentration of the coagulant whereas cohesiveness and springiness were not affected significantly. Sensory evaluation for appearance, colour, flavour, mouthfeel and overall acceptance of the selected samples showed no significant differences. Based on the higher fresh yield magnesium sulphate was found to be a better coagulant for soy-lupin tofu preparation.

Keywords

legumes; lupin; tofu; physicochemical; sensory; texture.

1 **Introduction**

2 Tofu, a very popular food in the Orient particularly Far Eastern countries, is traditionally made by
3 curding soybean milk using different coagulants. It is used as a meat substitute due to its high
4 protein contents with good balance of amino acids and better digestibility (Liu, 1999; Read,
5 2002). Due to its recognised nutritional benefits, there is an increase of tofu consumption among
6 the Western countries in recent years (Oboh, 2006).

7 Comparable in protein content (32% compared with 37% in soybean) and functional
8 properties yet lower in price, Australian Sweet Lupin (*Lupinus angustifolius* L.) is proved to be a
9 valuable alternative to soybean in many foods (Jayasena & Quail, 2004; Jayasena *et al.*, 2004). In
10 addition, lupin is lower in fat (6% compared with 18% in soybean) and higher in dietary fibre
11 contents (30% compared with 9% in soybean) that provides a healthier choice to be used as
12 ingredient in many foods (Hall & Johnson, 2004; Jayasena *et al.*, 2009). Compared to soybean,
13 pea and faba bean, lupin has lower antinutritional factors such as trypsin inhibitors that can
14 interfere with digestion, phytic acid which binds to minerals such as calcium and zinc thus
15 reducing the bioavailability, saponins and lectins that can act as gastric irritants (Hudson, 1994;
16 Petterson & Fairbrother, 1996). Research evidence suggests that lupin could be incorporated up
17 to 40% in the raw material of tofu making without any significant influence on physicochemical
18 and sensory qualities but the major concern was lower yield (Jayasena *et al.*, 2010). Protein is the
19 main functional component that determines the quantity and quality of tofu and studies have
20 shown that lupin protein and soy protein have similar functional properties (Jayasena *et al.*,
21 2004). Coagulation of the protein is the most important step in the tofu-making process as yield
22 and quality of tofu mostly depends on this step. Coagulation occurs due to cross linking of
23 protein molecules in the bean extract with the divalent cations.

1 Calcium sulphate and 'nigari' (sea water extract) are the most common coagulants used in
2 tofu manufacturing process (Shih *et al.*, 1997). However, recent studies have shown that calcium
3 lactate, magnesium sulphate and magnesium chloride are very effective coagulants for soy tofu
4 preparation (Prabhakaran *et al.*, 2006). The coagulation of soymilk depends on many factors
5 including variety of soybean, soymilk heating temperature and time, pH and coagulant type and
6 concentration (Hou & Chang, 2004). In addition, yield and quality of tofu have been reported to
7 be influenced by coagulants (Cai *et al.*, 1997; Poysa & Woodrow, 2004; Oboh, 2006). As with
8 type of coagulant, the concentration of the coagulant also affects the nature of tofu. The amount
9 of coagulant added in tofu manufacturing is one of the critical points as it determines the product
10 texture, flavour and yield (Wilson, 1995).

11 In the present study, coagulants such as calcium sulphate, calcium lactate, magnesium
12 sulphate and magnesium chloride have been utilised at different concentrations to find a
13 combination of coagulant type and concentration that could provide a better yield and quality of
14 soy-lupin tofu.

15

16 **Materials and methods**

17 **Materials**

18 Soybean was purchased from the local market (Perth). Australian Sweet Lupin flour was obtained
19 from Irwin Valley Pty Ltd, Australia. Calcium sulphate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and magnesium chloride
20 ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) were obtained from Ajax Finechem Pty Ltd Australia. Magnesium sulphate
21 (MgSO_4 anhydrous) was obtained from Mallinckrodt Chemicals, USA and calcium lactate
22 ($\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$) was obtained from VWR International.

23

1 **Preparation of soy-lupin tofu samples**

2 Soy-lupin tofu samples were prepared by replacing 30% soybean with lupin flour. The ratio of
3 70:30 soybean: lupin flour was chosen based on the studies by Jayasena *et al.* (2010) which
4 revealed that tofu prepared by replacing up to 40% of soybean with lupin bean (equal to around
5 30% lupin flour) had a quality equivalent to traditional tofu prepared solely from soybean. Soy-
6 lupin tofu samples were prepared according to the method developed by Jayasena *et al.* (2010).

7 A sample of 210 g soybean was washed and soaked in 630 ml water (bean: water, 1:3 w/v)
8 overnight for 16 hours. Soaked soybean and 90 g lupin flour was then blended with 3 L water
9 (1:10, dry bean + flour: water) using speed 2 of Breville 5 speed blender (Model BLR 50) for 2
10 min. The slurry was filtered through cheese cloth squeezed by hand pressure to obtain the extract
11 hereafter called “milk”. The milk was boiled for 5 minutes with occasional stirring followed by
12 cooling to 78 °C. The coagulant solution/suspension made by dissolving/mixing 9.0, 12.0 and
13 15.0 g (0.3, 0.4 and 0.5% w/v of the milk) calcium sulphate, calcium lactate, magnesium sulphate
14 or magnesium chloride in 20 ml distilled water was added and stirred properly. The coagulants
15 were soluble in 20 ml water at room temperature except calcium sulphate. To increase the
16 solubility of calcium sulphate, the suspension was prepared in 20 ml hot water. The mixture was
17 let stand for 30 minutes for coagulation. The coagulated curd was transferred to a mould (22 cm x
18 15 cm x 12.5 cm), drained off the whey fraction gravimetrically for about 5 minutes and pressed
19 for 45 minutes using a weight of 6 kg. The soy-lupin tofu was removed from the cheese cloth and
20 weighed immediately. Samples were taken for physicochemical analysis before storing in
21 distilled water at 5±1 °C. Samples were prepared in triplicate for each treatment. As the main
22 objective of the experiment was to study the effect of type and concentration of different
23 coagulants on the yield of soy-lupin tofu, the sample prepared by using 0.3% (w/v of milk)

1 calcium sulphate, the most commonly used coagulant type and concentration, was taken as a
2 control.

3 **Physicochemical analysis**

4 *Yield*

5 The tofu yield was calculated on the basis of the weight of pressed tofu obtained from 70:30
6 (w/w) soybean: lupin flour and expressed as g/100g raw material (bean+ flour). Dry tofu yield
7 was calculated by excluding the moisture content.

8

9 *Moisture, protein and fat contents*

10 AOAC (2000) methods were used to determine moisture (method 925.10), protein (method
11 950.36) and fat (method 963.15) contents of the tofu samples.

12

13 *Instrumental colour measurement*

14 Tofu colour was measured using Minolta spectrophotometer CM-508i (Minolta Co. Ltd.
15 Japan) and expressed as L^* (lightness), a^* ($+a^*$ = redness, $-a^*$ = greenness) and b^* ($+b^*$ =
16 yellowness, $-b^*$ = blueness) colour coordinates according to the methods specified by the
17 equipment manual. The instrument was equipped with a pulsed xenon arc lamp as light source, a
18 silicon photodiode array detector and has the illumination/measurement area of $\text{Ø}11\text{mm}$. The
19 instrument was calibrated using the white-coloured disc ($L^* = +98.82$, $a^* = -0.07$ and $b^* = -$
20 0.45) supplied with the instrument prior to the analysis. Three readings were recorded for each
21 sample.

1 *Texture profile analysis (TPA)*

2 Texture profile of the tofu samples was determined using TA-XT2i texture analyser (Stable
3 Micro System, Godalming, UK). A sample was obtained from the central portion of raw tofu and
4 cut into cubes (1.5 cm x 1.5 cm x 1.5 cm). A test speed of 1.0 mm/s and a 15 mm diameter
5 cylindrical probe was used for the analysis. The probe compressed the sample twice to 25% of its
6 original height by using a load cell of 5 kg. The TPA settings used were:

| | | |
|----|-------------------|-----------|
| 7 | Pre-test speed | : 2.0mm/s |
| 8 | Test speed | : 1.0mm/s |
| 9 | Post-test speed | : 1.0mm/s |
| 10 | Distance | : 25% |
| 11 | Time between bite | : 3s |

12
13
14 Three replicate tests were carried out for each tofu sample. The TPA curves were recorded and
15 used to calculate hardness, cohesiveness, springiness and chewiness (Bourne, 2002).

16 **Sensory evaluation**

17 A total of 53 semi trained panellists from the Curtin University's staff and students participated in
18 the sensory evaluation. Four soy-lupin tofu samples that included control sample and three other
19 high yielding samples (one out of each coagulant type) were selected for sensory evaluation
20 studies. Samples were cut into rectangular pieces (4 cm x 2 cm x 2 cm) and deep fried for 4 min
21 in commercially available vegetable oil. Tofu samples were placed in plastic cups which were
22 labelled with 3 digits random numbers. Panellists were also served with crackers and water for
23 the purpose to cleanse the palate between evaluations. A separate set of raw tofu samples
24 (without deep-frying) having different code numbers was used for colour evaluation. The sensory
25 attributes evaluated were appearance, colour, flavour, mouthfeel (oral texture) and overall
26 acceptance. The nine-point Hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 =

1 dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like
2 moderately, 8 = like very much, 9 = like extremely) was used for sensory evaluation.

3 **Statistical analysis**

4 The data collected for each of the physicochemical properties of different tofu samples were
5 analysed by analysis of variance (ANOVA) using SPSS for Window version 17.0. The
6 differences of means between groups were compared by using Tukey's honestly significant
7 difference (HSD) test. Since the results obtained from sensory evaluation were non-parametric,
8 the statistical analysis was conducted using Kruskal-Wallis test. Statistical significance was
9 established at $p \leq 0.05$.

10

11 **Results and discussion**

12 **Moisture content and yield (wet and dry)**

13 The moisture content remained same for different coagulant types and concentrations except for
14 0.5% magnesium sulphate that had a significantly higher value than the control (Table 1).
15 Coagulant concentration had shown no effect on moisture contents within a coagulant. However,
16 when the data were analysed on the basis of coagulant type (accumulating the effect of all three
17 concentrations) the samples prepared by using magnesium sulphate had significantly ($p \leq 0.05$)
18 higher moisture contents than the other coagulants (Table 2).

19 The fresh tofu yield ranged from 168-198 g/100g raw material for different coagulant type
20 and concentration combinations which was lower than the values reported in the previous studies
21 (Jayasena *et al.*, 2010). The difference could be due to the use of different soybean
22 samples/varieties. Soybean was purchased from the local retail market at about one year interval
23 and there was no information available on the variety or age of the beans. Whereas soybean

1 variety is one of the major factors that affects substantially on the tofu yield (Hou & Chang,
2 2004). Tofu yield was within the range of 150-200 g/100g soybean using Korean and Canadian
3 soybean cultivars as reported by Abd Karim *et al.* (1999), No and Meyers (2004), Noh *et al.*
4 (2005), Yoon and Kim (2007). In contrast Mujoo *et al.* (2003) reported tofu yield that ranged
5 from 269 to 343 g/100g bean for seven American soybean varieties.

6 Concentration of coagulants ranging from 0.3 to 0.5% (w/v of milk) had no significant effect
7 on fresh tofu yield. Similar results have been reported by earlier researchers (Prabhakaran *et al.*,
8 2006) that there was no effect on soy tofu yield when 0.4 or 0.5% (w/w of soy milk) of calcium
9 sulphate, calcium chloride, magnesium sulphate, calcium lactate or calcium acetate were used.

10 The magnesium sulphate delivered the highest fresh (wet) tofu yield among coagulants
11 (Table 2). It might be due to the higher moisture content of the samples prepared by using
12 magnesium sulphate since tofu yield and moisture contents are highly correlated (Cai *et al.*,
13 1997). This is probably due to the difference in gel network within the particles which is
14 influenced by different anions and their ionic strengths towards the water holding capacity of
15 protein gels (Obatolu, 2008).

16 The dry yield, on the other hand, demonstrated no significant difference for different
17 coagulants and their concentration (Table 1). This confirmed that the higher fresh yield delivered
18 by magnesium sulphate was due to higher moisture holding capacity of those samples.

19

20 **Colour**

21 Table 3 shows the values of L^* , a^* and b^* of soy-lupin tofu prepared by using different coagulant
22 types at different concentrations. The coagulant type and concentration had no significant effect
23 on L^* , a^* or b^* values of the samples.

1 Having a greater L^* value is a favourable characteristics as consumer prefer lighter or whiter
2 tofu (Hou & Chang, 2004) and good coagulant should produce a tofu with higher L^* value (Tay
3 *et al.*, 2006). The different coagulating agents used at different concentrations in this study were
4 found to have no significant effect ($p \leq 0.05$) on the colour of tofu. All of the samples had a
5 creamy white colour which is a desirable characteristic for good quality tofu. The results are in
6 agreement to those of Prabhakaran *et al.* (2006) who found no difference in the colour of
7 soybean tofu samples prepared by using 0.4-0.5% (w/v of soy milk) of calcium sulphate,
8 magnesium sulphate, magnesium chloride, calcium acetate and calcium lactate. However the
9 results are contrary to the study of Obatolu (2008) which revealed a significant difference in L^* ,
10 a^* and b^* colour coordinates of tofu samples prepared from Epsom salt, alum and lemon juice.
11 The difference in tofu colour in case of Obatolu (2008) might be due to the use of such
12 coagulants that had their own colour such as lemon juice that imparted a change in the tofu
13 colour.

14 **Fat and protein contents**

15 Coagulant concentration had an effect on the fat content of tofu but it depended upon the type of
16 coagulant (Table 4). There was no effect of concentration on the fat contents of tofu samples in
17 case of calcium salts (calcium sulphate and calcium lactate) whereas a significant decrease in fat
18 content with increase in coagulant concentration was determined in case of magnesium salts
19 (magnesium sulphate and magnesium chloride) (Table 4). The results are in agreement to those
20 of Cai and Chang (1998) who demonstrated a decrease in tofu fat content with an increase in
21 coagulant concentration. Although lower fat content may be preferable considering health
22 benefits, higher quantity of coagulant (0.5% w/v magnesium sulphate or magnesium chloride of
23 milk) is required to achieve the lower fat value.

1 Analysing the cumulative effect, samples prepared by using calcium lactate had the highest and
2 those prepared by using magnesium sulphate had the lowest fat contents (Table 5). This was most
3 probably be due to the release of fats during the tofu making process as some coagulants can
4 considerably decrease the fat binding capacity of the protein network formed during curding
5 (Obatolu, 2008).

6 The coagulant concentration had no significant effect on protein contents (Table 4). The
7 results are in agreement to previous studies (Cai & Chang, 1998). The protein content of lupin
8 containing tofu might be able to improve by optimizing the processing conditions especially
9 protein coagulation conditions. Zee *et al.* (1988) showed that faba bean having 24% less protein
10 contents than soybean produced a tofu with 50% more protein content than soy tofu. This was
11 mainly due to the reduction in protein loss during faba bean tofu preparation.

12 **Textural properties**

13 The textural properties of tofu play a critical role in tofu consumer acceptability (Sun & Breene,
14 1991; Hou & Chang, 2004). The textural properties of tofu samples prepared using different
15 types of coagulant at different concentrations demonstrated a variation in their textural properties
16 (Table 6). The hardness and chewiness of tofu samples demonstrated substantial changes with
17 different coagulants and their concentrations. The lowest hardness and chewiness was
18 demonstrated by the sample prepared by using 0.5% magnesium sulphate. There was no
19 significant difference in hardness among the samples prepared from calcium sulphate and
20 calcium lactate. Similarly, both cohesiveness and springiness were not significantly affected ($p \leq$
21 0.05) by the type and the concentration of coagulants.

22 In general soy-lupin tofu samples prepared by using calcium salts had higher hardness than
23 those prepared from magnesium salts (Table 7). It may probably be caused by the ability of these

1 salts to create a network structure with the protein molecules coming closer due to the loss of
2 water during coagulation (Obatolu, 2008).

3 Among the coagulants, tofu samples made by using magnesium salts (magnesium sulphate
4 and magnesium chloride) had lower hardness and chewiness than the samples prepared by using
5 calcium salts *i.e.* calcium sulphate and calcium acetate (Table 7). Previous researchers also found
6 that the tofu made by using 0.4-0.5% (w/v of soymilk) magnesium sulphate had lower hardness
7 than those prepared with the same concentrations of calcium sulphate, calcium acetate and
8 calcium lactate (Prabhakaran *et al.*, 2006). This may have link with the higher moisture content
9 of the samples prepared by using magnesium sulphate (Table 1). According to Wang and
10 Hesseltine (1982), cross-linking between protein molecules along with the presence of calcium
11 ions are required for soy protein coagulation. Magnesium ions can also be used instead of
12 calcium ions, since this divalent cation can form cross-linking between protein molecules.
13 However, the sites of cross-linking in the protein molecules may be different for both calcium
14 and magnesium causing the latter to form a loose network encompassing many air gaps within
15 the network. This might be a reason why magnesium sulfate is rarely used alone as a coagulant
16 for firm tofu preparation. It is commonly used along with other coagulants such as magnesium
17 chloride and calcium chloride. “Modified nigari” is a popular name used for such type of
18 coagulant mix (Hou *et al.*, 1997).

19 **Sensory evaluation**

20 The results for sensory evaluation are presented in Table 8. The scores for colour and appearance,
21 which are the first deciding factors that determine the acceptance or rejection of a product,
22 reflected that the tofu samples prepared by using different coagulants had a good acceptability.
23 Acceptance by colour and appearance were not affected by the use of different coagulants. All

1 samples had similar creamy white colour which is the acceptable colour for tofu (Hou & Chang,
2 2004).

3 Flavour, a combination of both taste and odour, was a concern in soy-lupin tofu as lupin has
4 a natural beany flavour. However, the results indicated that all of the samples had acceptable
5 flavour scores (Table 8). Besides lupin itself, the concentration and type of coagulant used could
6 determine the tofu flavour (Kao *et al.*, 2003). However in our study different coagulants had no
7 significant effect on the flavour of soy-lupin tofu. It is possible that deep frying could have
8 masked the beany flavour of the product.

9 Sensory acceptability of texture, which is perceived by touching and/or mouth feel, is an
10 important determinant of consumer acceptability of tofu (Obatolu, 2008). The results showed that
11 calcium salts had a greater coagulating power than magnesium salts causing them to produce a
12 better texture profile in terms of hardness and chewiness than that produced by magnesium salts
13 (Table 7). However sensory score for mouthfeel (oral texture) showed no significant difference
14 among the samples prepared by using different coagulants.

15 The evaluation of overall acceptability is important in determining how well a product is
16 accepted by consumers. Although a non-specific indication of the reasons, it is a good indication
17 of the potential consumer demand of the product. The overall acceptability of soy-lupin tofu
18 samples prepared by using different coagulants was not significantly different ($p \leq 0.05$) to the
19 control tofu sample. All samples had similar scores for overall acceptability. All samples received
20 ≥ 6 out of 9 scores for overall acceptability.

21

22 **Conclusions**

1 This study contributes to a wider variation of coagulant options such as calcium lactate,
2 magnesium sulphate and magnesium chloride to be used in the production of lupin incorporated
3 tofu. However, use of magnesium sulphate may be preferred as it produced a higher fresh (wet)
4 yield of lupin incorporated tofu. The market value of tofu depends on the yield and quality of
5 fresh produce. Since fresh soy-lupin tofu prepared by using magnesium sulphate produced a
6 softer product a combination of coagulants may be applied to get a better texture. However as
7 tofu is mostly consumed after deep frying which changes the texture of the product and masks
8 minor differences, a little softness of soy-lupin tofu prepared by using magnesium sulphate may
9 not effect on its acceptability and marketing.

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1 **Table 1** Moisture content and yield of soy-lupin tofu samples prepared by using different
 2 coagulant types and concentrations

| Coagulant | | Moisture (%) | Tofu Yield (g/100g raw material) | |
|--------------------|-------------------------------|---------------------------|-------------------------------------|-------------------------|
| Type | Concentration (% w/v of milk) | | Fresh | Dry |
| Calcium sulphate | 0.3 (control) | 75.4 ± 1.5 ^c | ^A 172 ± 6 ^c | 42.0 ± 1.1 ^a |
| | 0.4 | 75.6 ± 0.4 ^{bc} | ^A 173 ± 6 ^c | 42.1 ± 0.8 ^a |
| | 0.5 | 76.6 ± 0.6 ^{bc} | ^A 178 ± 7 ^{bc} | 41.4 ± 1.5 ^a |
| Calcium lactate | 0.3 | 77.1 ± 1.1 ^{abc} | ^A 180 ± 5 ^{abc} | 41.0 ± 0.8 ^a |
| | 0.4 | 76.4 ± 0.5 ^{bc} | ^A 175 ± 5 ^{bc} | 41.3 ± 0.5 ^a |
| | 0.5 | 75.3 ± 0.7 ^c | ^A 168 ± 4 ^c | 41.5 ± 1.0 ^a |
| Magnesium sulphate | 0.3 | 78.0 ± 0.6 ^{ab} | ^A 186 ± 8 ^{abc} | 40.8 ± 1.5 ^a |
| | 0.4 | 77.7 ± 0.2 ^{abc} | ^A 192 ± 3 ^{ab} | 42.8 ± 0.4 ^a |
| | 0.5 | 79.5 ± 0.7 ^a | ^A 198 ± 5 ^a | 40.6 ± 1.6 ^a |
| Magnesium chloride | 0.3 | 76.4 ± 0.3 ^{bc} | ^A 179 ± 3 ^{bc} | 42.0 ± 0.2 ^a |
| | 0.4 | 77.4 ± 0.4 ^{abc} | ^A 182 ± 7 ^{abc} | 41.0 ± 0.8 ^a |
| | 0.5 | 76.8 ± 0.8 ^{bc} | ^A 180 ± 6 ^{abc} | 41.8 ± 0.6 ^a |

3 Means (± SEM; n = 3) with different superscripts (in lower case) within the same column are significantly different
 4 (p ≤ 0.05). Means with similar superscripts (in upper case) in the fresh yield column show a non significant difference
 5 (p ≤ 0.05) within a coagulant type.
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 8 **Table 2** Effect of different coagulants (cumulative effect) on the moisture content and yield of
 9 soy-lupin tofu samples

| Coagulant | Moisture (%) | Wet yield (g/100g raw material) | Dry yield (g/100g raw material) |
|--------------------|-------------------------|---------------------------------|---------------------------------|
| Calcium sulphate | 75.9 ± 1.5 ^b | 173.8 ± 9.7 ^b | 41.8 ± 1.1 ^a |
| Calcium lactate | 76.3 ± 1.5 ^b | 174.5 ± 8.9 ^b | 41.3 ± 1.2 ^a |
| Magnesium sulphate | 78.4 ± 1.1 ^a | 191.9 ± 9.2 ^a | 41.4 ± 2.2 ^a |
| Magnesium chloride | 76.8 ± 0.9 ^b | 180.4 ± 8.4 ^b | 41.6 ± 0.9 ^a |

10 Means (± SEM; n = 3) with different superscripts (in lower case) within the same column are significantly
 11 different (p ≤ 0.05).
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1 **Table 3** Colour analysis of soy-lupin tofu samples prepared from different coagulant types and
 2 concentrations

| Coagulant | | Colour coordinates | | |
|--------------------|----------------------------------|--------------------|-------------------|------------------|
| Type | Concentration (% w/v of milk) | L^* | a^* | b^* |
| Calcium sulphate | 0.3 (control) | 85.8 ± 0.3^a | 0.24 ± 0.12^a | 16.7 ± 0.1^a |
| | 0.4 | 86.5 ± 0.1^a | 0.26 ± 0.11^a | 16.4 ± 0.4^a |
| | 0.5 | 86.6 ± 0.1^a | 0.13 ± 0.05^a | 16.9 ± 0.3^a |
| Calcium lactate | 0.3 | 87.0 ± 0.4^a | 0.16 ± 0.02^a | 17.1 ± 0.3^a |
| | 0.4 | 86.7 ± 0.1^a | 0.14 ± 0.03^a | 16.8 ± 0.3^a |
| | 0.5 | 86.3 ± 0.4^a | 0.11 ± 0.04^a | 17.0 ± 0.3^a |
| Magnesium sulphate | 0.3 | 87.1 ± 0.1^a | 0.20 ± 0.08^a | 17.0 ± 0.2^a |
| | 0.4 | 87.3 ± 0.1^a | 0.19 ± 0.03^a | 16.7 ± 0.1^a |
| | 0.5 | 87.5 ± 0.2^a | 0.21 ± 0.09^a | 16.7 ± 0.2^a |
| Magnesium chloride | 0.3 | 86.4 ± 0.5^a | 0.21 ± 0.08^a | 17.1 ± 0.5^a |
| | 0.4 | 87.1 ± 0.2^a | 0.22 ± 0.10^a | 17.2 ± 0.4^a |
| | 0.5 | 87.0 ± 0.2^a | 0.19 ± 0.08^a | 17.1 ± 0.3^a |

3 Means (\pm SEM; n = 3) with similar superscripts in a column are not significantly different ($p \leq 0.05$).
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1 **Table 4** Fat and protein contents of soy-lupin tofu samples from various coagulant types and
 2 concentrations

| Coagulant | | Fat (g/100g db) | Protein (g/100g db) |
|--------------------|----------------------------------|---------------------------------------|-------------------------|
| Type | Concentration (% w/v of milk) | | |
| Calcium sulphate | 0.3 (control) | ^A 23.8 ± 0.9 ^{ab} | 55.0 ± 0.5 ^a |
| | 0.4 | ^A 23.4 ± 0.7 ^{ab} | 53.8 ± 1.1 ^a |
| | 0.5 | ^A 22.9 ± 0.9 ^{ab} | 53.2 ± 0.5 ^a |
| Calcium lactate | 0.3 | ^A 25.6 ± 0.4 ^a | 55.3 ± 0.4 ^a |
| | 0.4 | ^A 24.3 ± 0.7 ^a | 54.9 ± 0.3 ^a |
| | 0.5 | ^A 24.2 ± 1.0 ^a | 56.5 ± 1.2 ^a |
| Magnesium sulphate | 0.3 | ^A 22.9 ± 0.4 ^{ab} | 55.4 ± 0.4 ^a |
| | 0.4 | ^B 20.1 ± 1.2 ^{bc} | 55.3 ± 0.6 ^a |
| | 0.5 | ^C 16.8 ± 0.6 ^c | 54.9 ± 0.8 ^a |
| Magnesium chloride | 0.3 | ^A 26.0 ± 0.3 ^a | 54.8 ± 1.1 ^a |
| | 0.4 | ^B 22.4 ± 1.4 ^{ab} | 55.3 ± 0.2 ^a |
| | 0.5 | ^C 17.9 ± 0.4 ^c | 55.8 ± 0.2 ^a |

3 Means (± SEM; n = 3) with different superscripts (in lower case) within the same column are significantly
 4 different (p ≤ 0.05). Means with different superscripts (in upper case) in the fat content column show a
 5 significant difference (p ≤ 0.05) within a coagulant type.
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 8 **Table 5** Effect of coagulant type (cumulative effect) on the fat and protein content of soy-lupin
 9 tofu samples

| Coagulant | Fat (g/100g db) | Protein (g/100g db) |
|--------------------|-------------------------|-------------------------|
| Calcium sulphate | 23.4 ± 1.3 ^b | 54.0 ± 0.7 ^a |
| Calcium lactate | 24.7 ± 1.2 ^a | 55.6 ± 0.9 ^a |
| Magnesium sulphate | 20.0 ± 2.9 ^c | 55.2 ± 0.8 ^a |
| Magnesium chloride | 22.1 ± 3.7 ^b | 55.3 ± 0.5 ^a |

10 Means (± SEM; n = 3) with different superscripts within the same column are significantly different
 11 (p ≤ 0.05).
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1 **Table 6** Textural properties of soy-lupin tofu samples made from different coagulant types and
 2 concentrations

| Coagulant | | Hardness (g) | Cohesiveness | Springiness (cm) | Chewiness (g.cm) |
|--------------------|-------------------------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|
| Type | Concentration (% w/v of milk) | | | | |
| Calcium sulphate | 0.3 (control) | 348.1 ± 56.2 ^a | 0.81 ± 0.02 ^a | 0.88 ± 0.03 ^a | 246.1 ± 37.0 ^a |
| | 0.4 | 310.9 ± 12.7 ^{ab} | 0.80 ± 0.01 ^a | 0.88 ± 0.02 ^a | 218.7 ± 8.3 ^{ab} |
| | 0.5 | 251.5 ± 14.3 ^{abc} | 0.80 ± 0.01 ^a | 0.87 ± 0.04 ^a | 173.5 ± 7.7 ^{bcd} |
| Calcium lactate | 0.3 | 255.4 ± 10.6 ^{abc} | 0.81 ± 0.03 ^a | 0.85 ± 0.04 ^a | 175.4 ± 5.5 ^{abcd} |
| | 0.4 | 268.3 ± 4.9 ^{abc} | 0.80 ± 0.01 ^a | 0.88 ± 0.03 ^a | 187.9 ± 2.1 ^{abcd} |
| | 0.5 | 307.9 ± 20.9 ^{ab} | 0.80 ± 0.01 ^a | 0.87 ± 0.03 ^a | 214.1 ± 17.0 ^{abc} |
| Magnesium sulphate | 0.3 | 175.9 ± 10.3 ^{cd} | 0.81 ± 0.01 ^a | 0.91 ± 0.05 ^a | 128.2 ± 3.6 ^{de} |
| | 0.4 | 201.5 ± 5.2 ^{cd} | 0.80 ± 0.01 ^a | 0.86 ± 0.04 ^a | 147.4 ± 6.6 ^{cde} |
| | 0.5 | 133.7 ± 16.5 ^d | 0.79 ± 0.01 ^a | 0.86 ± 0.04 ^a | 91.1 ± 11.9 ^d |
| Magnesium chloride | 0.3 | 194.0 ± 15.4 ^{cd} | 0.80 ± 0.01 ^a | 0.90 ± 0.03 ^a | 133.3 ± 12.3 ^{de} |
| | 0.4 | 235.7 ± 15.0 ^{bcd} | 0.80 ± 0.01 ^a | 0.84 ± 0.03 ^a | 167.1 ± 11.7 ^{bcd} |
| | 0.5 | 207.9 ± 0.4 ^{bcd} | 0.80 ± 0.01 ^a | 0.88 ± 0.04 ^a | 139.4 ± 6.4 ^{de} |

3 Means (± SEM; n = 3) with different superscripts within the same column are significantly different (p ≤ 0.05).

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5 **Table 7** Effect of different coagulants (cumulative effect) on the textural properties of soy-lupin
 6 tofu samples

| Coagulant | Hardness (g) | Cohesiveness | Springiness (s) | Chewiness (s) |
|--------------------|---------------------------|--------------------------|--------------------------|---------------------------|
| Calcium sulphate | 303.4 ± 66.4 ^a | 0.80 ± 0.01 ^a | 0.88 ± 0.03 ^a | 278.3 ± 67.3 ^a |
| Calcium lactate | 277.3 ± 31.6 ^a | 0.80 ± 0.02 ^a | 0.87 ± 0.03 ^a | 256.7 ± 30.6 ^a |
| Magnesium sulphate | 188.5 ± 18.8 ^b | 0.81 ± 0.01 ^a | 0.91 ± 0.04 ^a | 167.7 ± 18.0 ^b |
| Magnesium chloride | 192.8 ± 44.2 ^b | 0.80 ± 0.01 ^a | 0.86 ± 0.04 ^a | 177.4 ± 39.8 ^b |

7 Means (± SEM; n = 3) with different superscripts within the same column are significantly different (p ≤ 0.05).

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1 **Table 8** Sensory evaluation of soy-lupin tofu prepared by using different coagulant types and
 2 concentrations

| Coagulant | | Appearance | Colour | Flavour | Mouthfeel | Overall acceptability |
|--------------------|-------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Type | Concentration (% w/v of milk) | | | | | |
| Calcium sulphate | 0.3% (control) | 6.1 ± 0.2 ^a | 6.4 ± 0.2 ^a | 5.9 ± 0.2 ^a | 5.9 ± 0.2 ^a | 6.2 ± 0.2 ^a |
| Calcium lactate | 0.3% | 6.2 ± 0.2 ^a | 6.3 ± 0.2 ^a | 6.3 ± 0.2 ^a | 5.8 ± 0.2 ^a | 6.3 ± 0.2 ^a |
| Magnesium sulphate | 0.5% | 6.2 ± 0.2 ^a | 6.3 ± 0.1 ^a | 5.9 ± 0.2 ^a | 5.7 ± 0.2 ^a | 6.1 ± 0.2 ^a |
| Magnesium chloride | 0.4% | 6.3 ± 0.1 ^a | 6.3 ± 0.2 ^a | 5.8 ± 0.2 ^a | 5.6 ± 0.2 ^a | 6.0 ± 0.2 ^a |

3 Means (± SEM; n = 53) with different superscripts within the same column are significantly different (p ≤ 0.05).