Yogurt fortification with predigested germinated whole soy powder for enhanced therapeutic benefits

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Introduction:

- **Foods such as cow's milk and soy milk and their derivatives contain compounds that may be made biologically active via fermentation or enzyme treatments**

- **These compounds may provide benefits beyond basic nutrition**

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Introduction, cont.:

- Controversy exists regarding the bioavailability and metabolism of isoflavones, their health benefits and adverse effects
- Isoflavone content of soybeans increase upon germination and fermentation
- Germination of soybeans also improves 'beany' odor and flavor, deactivates trypsin inhibitors

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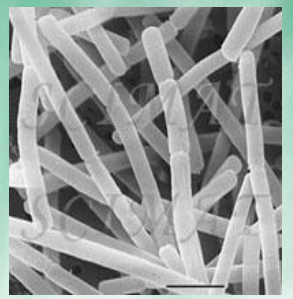
Introduction, cont.:



- Data accumulating on the health benefits of probiotic organisms
- Dairy foods such as yogurt are considered excellent carriers of probiotics

- Cow's milk and soy milk have promising future as nutraceutical foods, our aim was to combine the health benefits of the two foods

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Objectives:

- Incorporate whole predigested and germinated whole soybean powder into cow's milk substrate to increase the yields of biologically active compounds in yogurt blend to meet the recommended requirements for added health claims.

(Recommended daily requirement by FDA for soy protein is 6.25g per serving; 25g soy protein/day; suggested recommendation: 44-50mg isoflavone/day for health benefits).

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Specific objectives:

- Production of predigested and germinated whole soy pwder with increased concentration of isoflavones and reduced oligosaccharide (stachyose)

- Determine growth and activity of lactic acid bacteria and a probiotic organism in reconstituted germinated whole soy powder (GSP), non-germinated whole soy powder (NGSP) and non-fat dry milk (NFDM) + GSP or NGSP

- Develop yogurt from blends of cow's milk and whole soymilk base for consumer acceptance

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Specific objectives, cont.:

- Determine the effect of processing and refrigerated storage on isoflavone and stachyose contents of yogurt fortified with non-germinated and germinated (predigested) whole soy powder

- Shelf life studies and viability of whole soy-fortified yogurts stored at 4 ºC

- Overall goal: Conferment of better health benefits to consumers by providing bioactive compounds from both soy and cow's milk in form of yogurt.

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Manufacture of predigested and germinated whole soybean powder:

- Soybeans varieties used: **V**inton 81 (V81), E05276-T (ET) and DF 222 were utilized for this study.
- Predigested, germinated soy powder (GSP) preparation: An optimized process (patent #US 7,067, 163 B2) was used.

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Predigested, germinated whole soybean powder (GSP) preparation (US Patent #7,067,163 B2)

Wash and rinse twice 5kg low fat soy beans in tap water

⇩

Acidify water with citric acid to pH 4.8

⇩

Steep in distilled water @ 24-28°C for 14-16h

⇩

Incubate in plastic totes, slightly ajar (3-5cm) @24-28°C for 18-24h
(Partially hydrolyzed soybeans-germination)

⇩

Deacidify with 5x vol/wt of water @ 45°C for 1h or repeat re-soak step (rehydration)

⇩

Mechanically dehull and mill
Gelatinize @ 92°C for 15 min and cool to 60°C

⇩

Homogenize @ 3000 and 12,000 psi

⇩

Spray dry (germinated soy powder, GSP)

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Predigested, germinated whole soybean powder (GSP) preparation:

Germinated soybeans

Wet dehulling

Wet milling

Predigested, germinated whole soybean powder (GSP) preparation, cont.:

Homogenization

Ultra-homogenization

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Predigested, germinated whole soybean powder (GSP) preparation, cont.:

Spray drying

Dried powder

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Table 1. Compositional analysis of the predigested, germinated whole soybean powders:

Sample	Protein (%)	Fat (%)	Carbohydrate (%)	Dry Matter (%)	Ash (%)	Neutral Detergent Fiber (%)
[1]GV 81	56.03 ± 0.35[a]	20.85 ± 0.01[c]	18.80 ± 0.30[d]	92.41 ± 0.01[b]	4.32 ± 0.63[b]	4.54 ± 0.05[a]
NGV 81	50.76 ± 0.49[b]	18.39 ± 0.01[b]	26.06± 0.65[ab]	92.17 ± 0.28[b]	4.79 ± 0.16[ab]	4.39 ± 0.04[a]
GDF 222	51.00 ± 0.68[b]	22.40 ± 0.44[d]	22.20 ± 0.36[c]	92.25 ± 0.15[b]	4.40 ± 0.14[b]	4.55 ± 0.04[a]
NGDF 222	50.28 ± 0.95[b]	17.62 ± 0.05[b]	27.81± 0.88[a]	93.22 ± 0.15[a]	5.09 ± 0.02[a]	4.39 ± 0.09[a]
GET	54.97 ± 0.03[a]	15.81 ± 0.13[a]	24.20 ± 0.26[bc]	92.60 ± 0.13[ab]	5.02 ± 0.16[a]	3.84 ± 0.02[b]

[a-d] Means in the same column with small letter superscripts are significantly different ($p<0.05$); n = 2 for all samples

[1]GV 81 = Germinated Vinton 81
NGV 81 = Non-germinated Vinton 81
GDF 222 = Germinated DF 222
NGDF 222 = Non-germinated DF 222
GET = Germinated E05276-T

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Table 2. Total isoflavone contents of whole soy bean powders produced (µg/g):

Seed treatment	Soybean varieties		
	Vinton 81	DF 222	E05276-T
Untreated	378.74[c]	375.33[c]	290.13[b]
Predigested	509.41[a]	726.16[a]	N/A
Germinated	419.84[b]	414.77[b]	611.87[a]

(N=3; $p<0.05$)

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Table 3. Stachyose contents of whole soybean powders produced (mg/g):

	Soybean varieties		
Seed treatment	Vinton 81	DF 222	E05276-T
Untreated	41.35^{b}	46.65^{a}	N/A
Germinated	19.15^{c}	15.45^{cd}	13.90^{d}

(N=2; $p<0.05$)

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Low-fat soy fortified yogurt treatments:

- **Soy fortified yogurts were formulated with the following soy powders as screened by a trained panel:**

  GV 81
  NGV 81
  GDF 222
  NGDF 222

- **Based on the above selection the following soy fortified yogurts were manufactured:**

252 = GV 81 + NFDM **169 = NGV81 + NFDM**
344 = GDF 222 + NFDM **159 = NGDF 222 + NFDM**
894 = NGV 81(control) **949 = NFDM(control)**

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Formulations of low-fat soy-fortified yogurts (Swiss-style): :

Ingredients (%)	GV 81	NGV 81	GDF 222	NGDF 222	GET
Soy powder	7.58	7.59	7.58	7.51	7.56
NFDM	7.00	7.00	7.00	7.00	7.00
Sucrose	7.00	7.00	7.00	7.00	7.00
Stabilizer	0.50	0.50	0.50	0.50	0.50
Strawberry puree	13.00	13.00	13.00	13.00	13.00
Added water	64.92	64.91	64.92	64.99	64.94
Total	100.00	100.00	100.00	100.00	100.00

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Flow diagram for manufacture of low-fat soy-fortified yogurts (Swiss-style):

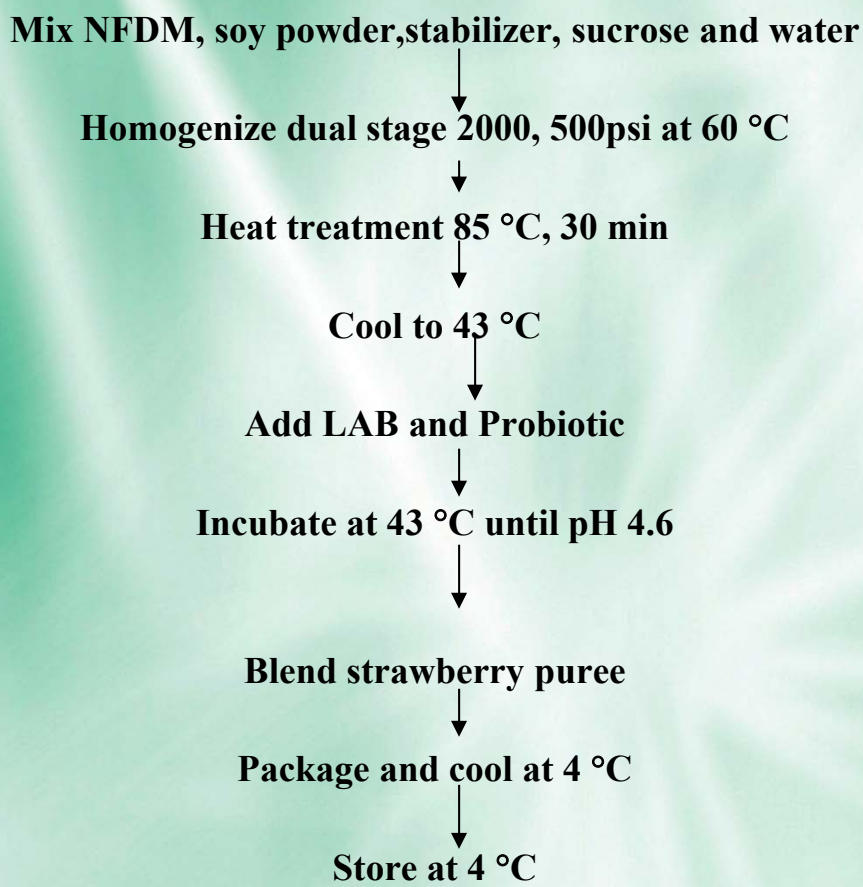

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Table 4. Compositional analysis of soy-fortified yogurts:

Sample	Protein (%)	Fat (%)	CHO (%)	Dry matter (%)	Ash (%)	Dietary Fiber (%)
252	5.98^{b}	0.98^{a}	13.99^{d}	24.92^{a}	3.02^{b}	0.92^{b}
169	5.87^{b}	1.13^{a}	13.62^{d}	24.73^{a}	3.12^{b}	0.74^{b}
344	5.16^{cd}	0.14^{bc}	17.78^{b}	25.63^{a}	2.58^{bc}	0.74^{b}
159	5.08^{d}	0.46^{b}	17.49^{b}	25.21^{a}	2.20^{c}	0.54^{c}
817	6.82^{a}	0.40^{b}	19.39^{a}	26.52^{a}	2.92^{b}	0.63^{bc}
894	4.80^{d}	1.32^{a}	15.72^{c}	26.91^{a}	2.08^{c}	1.60^{a}
949	5.56^{bc}	0.00^{c}	17.75^{b}	26.40^{a}	4.09^{a}	0.00^{d}

N = 2; $p<0.05$

252 = GV 81 + NFDM ; 169 = NGV81 + NFDM
344 = GDF 222 + NFDM ; 159 = NGDF 222 + NFDM
894 = NGV 81(control); 949 = NFDM(control)

Table 12. pH of soy-fortified yogurt samples during prolonged cold storage at 4 °C: $p < 0.05$

Weeks of storage	252	169	344	159	894	949
1	4.56^{aC}	4.59^{aC}	4.39^{aA}	4.49^{aB}	4.67^{aD}	4.41^{aA}
2	4.56^{aC}	4.60^{aC}	4.34^{aA}	4.49^{aB}	4.69^{aD}	4.37^{aA}
3	4.56^{aB}	4.62^{aC}	4.46^{cA}	4.64^{bC}	4.75^{bD}	4.47^{bA}
4	4.64^{aB}	4.67^{aBC}	4.43^{bcA}	4.62^{bC}	4.84^{cD}	4.46^{bA}
5	4.56^{aC}	4.63^{aD}	4.37^{abA}	4.60^{bCD}	4.76^{bE}	4.44^{abB}
6	4.67^{bB}	4.79^{bC}	4.68^{dB}	4.49^{aA}	4.82^{cC}	4.55^{cA}

N=3; p<0.05

252 = GV81+ NFDM
169 = GV81+NFDM
344 =GDF222+NFDM
159 = NGDF 222 + NFDM
894 = NGV 81(control)
949 =NFDM(control)

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Table 5. Overall acceptability of soy fortified yogurts as determined by a consumer panel (n=112)

Attribute	252	169	949	344	159	894	P-value	Sig
Appearance	6.80^{ab}	6.80^{ab}	6.57^{cb}	6.96^{ab}	7.01^{a}	6.24^{c}	0.0001	***
Body texture	6.34^{ab}	6.07^{ab}	6.51^{a}	6.25^{ab}	6.40^{a}	5.81^{b}	0.0064	**
Flavor	5.43^{a}	5.21^{a}	5.58^{a}	5.39^{a}	5.67^{a}	4.43^{b}	0.0001	***
Overall Acceptance	5.48^{a}	5.32^{a}	5.71^{a}	5.59^{a}	5.72^{a}	4.55^{b}	0.0001	***

252 = GV 81 + NFDM ; 169 = NGV81 + NFDM
344 = GDF 222 + NFDM ; 159 = NGDF 222 + NFDM
894 = NGV 81(control); 949 = NFDM(control)

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Table 6. Effect of refrigerated storage total isoflavone concentrations in yogurts fortified with germinated or non-germinated soybean powders (µg/g):

Yogurt samples	1st Week	6th Week	Percent increase
252	131.20^{cdB}	161.30^{cA}	18.7
169	149.18^{cB}	230.91^{bA}	35.4
344	89.68^{dB}	128.50^{cA}	30.2
159	197.95^{bB}	259.82^{bA}	23.8
817	201.95^{b}	N/A	N/A
894	377.15^{aB}	478.66^{aA}	21.2
949	0.0^{eA}	0.0^{dA}	0.0

N=2; $p<0.05$

252 = GV 81 + NFDM ; 169 = NGV81 + NFDM
344 = GDF 222 + NFDM ; 159 = NGDF 222 + NFDM
894 = NGV 81(control); 949 = NFDM(control)

Table 7. Stachyose contents of yogurts fortified with germinated or non-germinated whole soy powders.

Treatments (Yogurt samples)	Stachyose (mg/g)
252	4.41[d]
169	10.04[b]
344	3.40[e]
159	8.45[c]
817	2.82[f]
894	17.25[a]

252 = GV 81 + NFDM
169 = NGV81 + NFDM
344 = GDF 222 + NFDM
159 = NGDF 222 +NFDM
894 = NGV 81(control);
949 = NFDM(control)

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Table 8. Reduction of stachyose in yogurts manufactured with germinated and non-germinated soy powders:

Soy powder	Yogurt sample	% Stachyose reduced
GV 81 (9.58 mg/g)	252 (4.41 mg/g)	54.0
NGV 81 (20.68 mg/g)	169 (10.04 mg/g)	51.5
NGV 81 (41.35 mg/g)	894 (17.25 mg/g)	58.3
GDF 222 (7.73 mg/g)	344 (3.40 mg/g)	56.0
NGDF 222 (23.33 mg/g)	159 (8.45 mg/g)	63.8
GET (6.95 mg/g)	817 (2.82 mg/g)	59.4

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Table 9. Viability of *Lactobacillus delbreuckii* subsp.*bulgaricus* (CFU/g) during six weeks of storage at 4 °C

Weeks of storage	252	169	344	159	894	949
1	5.57×10^{7b}	4.47×10^{7b}	5.88×10^{7c}	5.47×10^{7a}	2.27×10^{7bc}	5.01×10^{7a}
2	6.31×10^{7b}	7.49×10^{7ab}	7.90×10^{7b}	4.61×10^{7a}	4.45×10^{7ab}	6.69×10^{7a}
3	6.73×10^{7ab}	6.80×10^{7ab}	1.02×10^{8ab}	5.63×10^{7a}	4.60×10^{7a}	6.37×10^{7a}
4	8.27×10^{7ab}	7.77×10^{7a}	1.17×10^{8a}	8.26×10^{7a}	4.17×10^{7ab}	6.43×10^{7a}
5	9.50×10^{7a}	8.00×10^{7a}	9.90×10^{7ab}	7.00×10^{7a}	4.60×10^{7a}	6.60×10^{7a}
6	6.47×10^{7b}	9.07×10^{7a}	8.37×10^{7bc}	6.57×10^{7a}	2.13×10^{7c}	5.87×10^{7a}

N=3; $p<0.05$

252 = GV 81 + NFDM
169 = NGV81 + NFDM
344 = GDF 222 + NFDM
159 = NGDF 222 + NFDM
894 = NGV 81(control)
949 = NFDM(control)

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Table 10. Viability of *Streptococcus thermophilus* (CFU/g) during six weeks of storage at 4 °C

Weeks of storage	*252	169	344	159	894	949
1	$6.97x10^{7ab}$	$6.71x10^{7ab}$	$6.80x10^{7c}$	$6.03 x10^{7a}$	$2.98x10^{7a}$	$2.39x10^{7b}$
2	$5.49 x10^{7b}$	$6.82x10^{7ab}$	$6.87x10^{7bc}$	$6.04 x10^{7a}$	$3.69x10^{7a}$	$7.35x10^{7a}$
3	$8.63x10^{7ab}$	$5.60x10^{7b}$	$1.03x10^{8a}$	$8.50 x10^{7a}$	$4.83 x10^{7a}$	$6.83 x10^{7a}$
4	$8.83x10^{7a}$	$9.27x10^{7a}$	$9.93x10^{7ab}$	$6.53x10^{7a}$	$3.37x10^{7a}$	$6.33 x10^{7a}$
5	$7.63x10^{7ab}$	$6.97x10^{7ab}$	$8.80x10^{7bc}$	$6.53 x10^{7a}$	$3.83 x10^{7a}$	$6.07 x10^{7a}$
6	$6.80x10^{7ab}$	$8.20x10^{7ab}$	$1.00x10^{8ab}$	$8.10 x10^{7a}$	$3.00 x10^{7a}$	$7.07 x10^{7a}$

n=3; $p<0.05$

252 = GV 81 + NFDM
169 = NGV81 +NFDM
344 = GDF 222 + NFDM
159 = NGDF 222 + NFDM
894 = NGV 81(control);
949 = NFDM(control)

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Table 11. Viability of *Lactobacillus acidophilus* NCFM (CFU/g) during six weeks of storage at 4 ºC

Weeks of storage	*252	169	344	159	894	949
1	$4.69x10^{7b}$	$3.48 x10^{7b}$	$4.71 x10^{7c}$	$4.21 x10^{7c}$	$1.97x10^{7c}$	$3.72x10^{7a}$
2	$5.39x10^{7ab}$	$6.31x10^{7a}$	$5.79x10^{7bc}$	$5.60x10^{7bc}$	$2.92x10^{7bc}$	$5.01x10^{7a}$
3	$6.87x10^{7ab}$	$6.73x10^{7a}$	$6.97x10^{7b}$	$7.07x10^{7ab}$	$2.40x10^{7bc}$	$5.23x10^{7a}$
4	$7.73x10^{7ab}$	$7.06x10^{7a}$	$8.50x10^{7ab}$	$8.03 x10^{7a}$	$3.60x10^{7abc}$	$4.73x10^{7a}$
5	$8.80 x10^{7a}$	$7.63x10^{7a}$	$9.47x10^{7a}$	$7.83x10^{7ab}$	$4.87 x10^{7a}$	$5.80x10^{7a}$
6	$7.43x10^{7ab}$	$8.43 x10^{7a}$	$9.43x10^{7a}$	$7.00x10^{7ab}$	$4.17 x10^{7ab}$	$5.07x10^{7a}$

N=3; $p<0.05$

252 = GV 81 + NFDM
169 = NGV81 + NFDM
344 = GDF 222 + NFDM
159 = NGDF 222 + NFDM
894 = NGV 81(control);
949 = NFDM(control)

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Table 12. pH of soy-fortified yogurt samples during prolonged cold storage at 4 °C: $p < 0.05$

Weeks of storage	252	169	344	159	894	949
1	4.56^{aC}	4.59^{aC}	4.39^{aA}	4.49^{aB}	4.67^{aD}	4.41^{aA}
2	4.56^{aC}	4.60^{aC}	4.34^{aA}	4.49^{aB}	4.69^{aD}	4.37^{aA}
3	4.56^{aB}	4.62^{aC}	4.46^{cA}	4.64^{bC}	4.75^{bD}	4.47^{bA}
4	4.64^{aB}	4.67^{aBC}	4.43^{bcA}	4.62^{bC}	4.84^{cD}	4.46^{bA}
5	4.56^{aC}	4.63^{aD}	4.37^{abA}	4.60^{bCD}	4.76^{bE}	4.44^{abB}
6	4.67^{bB}	4.79^{bC}	4.68^{dB}	4.49^{aA}	4.82^{cC}	4.55^{cA}

N=3; p<0.05

252 = GV81+ NFDM
169 = GV81+NFDM
344 =GDF222+NFDM
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894 = NGV 81(control)
949 =NFDM(control)

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CONCLUSIONS

- **Fortification of milk bases with whole soymilk or powder for fermented products will enhance bioactive compounds and viability of probiotics, hence increases possible health benefits.**
- **Sensory evaluation showed that soy fortified yogurt is acceptable to consumers.**
- **Soaking and/or germination increased isoflavone contents and decreased stachyose concentrations in all soybean varieties.**
- **Whole soy-fortified yogurts had the highest viability of lactic acid bacteria/probiotic, substantial amount of isoflavones, high protein and high dietary fiber.**

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Acknowledgments:

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Thank you!

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Questions???

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