Microstructure and adsorption properties of gelatinized-tapioca starch beads modified by freezing and a freeze-drying method

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Rattanapitigorn, P., Raviyan, P., Khanongnuch, C., Ozimek, L. and Leesawat, P. (2005). Microstructure and adsorption properties of gelatinized-tapioca starch beads modified by freezing and a freeze-drying method. Journal of Agricultural Technology 1(2): 267-278.

The effects of two freezing methods: slow freezing (SF) at -20°C for 24 hours, and quick freezing (QF) at -176°C for 5-10 min and three commercial brands of Golden Chef®, Special Sacoo® or Thaiworld® on moisture content, microstructure, porosity, total bulk volume, adsorption properties (initial adsorption, adsorption capacity and adsorption behaviour) and gel strength of freeze-dried-gelatinized tapioca starch beads (FDTB) were studied in order to determine the potential use of FDTB as a carrier for immobilizing probiotic bacteria. The SF-FDTB was prepared by gelatinizing tapioca starch beads, slow-freezing and freeze-drying, respectively. The QF-FDTB was prepared by gelatinizing tapioca starch beads, quick-freezing and freeze-drying, respectively. The SF-FDTB provided the porous beads with large pore size (ca 57.04 μm, diam.) whereas the QF-FDTB provided the beads with smaller pore size (ca 11.18 μm, diam.). The adsorption property of SF- and QF-FDTB was affected by the pore size and surface area of the beads. The sponge-like texture of the SF-FDTB promotes the rapid adsorption into the beads when compared to that of the QF-FDTB. The firm texture was detected from the Golden Chef® FDTB processed by quick freezing and slow freezing.

Keywords: adsorption, freeze drying, tapioca starch beads, porosity

Introduction

Tapioca starch beads (TSB) or tapioca starch pearls are recognized as food ingredients in Thailand and many countries in Asia. Tapioca starch beads are made from the tapioca starch (*Manihot esculenta* Crantz). Tapioca starch

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has been applied over a wide range of products in many industries, for example, foods, pharmaceuticals, and textiles, either as a raw material or as an additive. In the food industry, tapioca starch has been used as a thickener, gelling agent, bulking agent, anti-stick agent, and raw material for fermentation (Whistler *et al*., 1984; Swinkels, 1985). The commercial production of TSB involves: adjusting the moisture content of tapioca starch powder to the level that the starch molecule could be attached to each other, forming the bead in the rolling bowl, drying and size-screening (Thaiwa Co. Ltd., Bangkok, Thailand, unpublished data).

Freezing is a method in which the temperature of a material is reduced below its freezing point and a proportion of the water undergoes a change in state to form ice crystals. The growing of ice crystal depends on the rate of freezing. In fast freezing, smaller ice crystals form within both cells and intercellular spaces when compared to that of the slow freezing. Freeze-drying is a method of drying food or pharmaceuticals or tissue. The material is frozen and then warmed in a vacuum so that the ice sublimes. Freeze-drying provided the dried-porous product. Freeze-drying has been used to modify the structure of the biopolymer beads, especially chitosan and alginate beads for using in many biotechnological purposes, such as water denitrification, matrices for the immobilization of denitrifying isolates, carriers of bacteria or spores for biological control of soil-borne root diseases, and carriers of Gram positive lactic acid bacteria starter cultures involved in dairy and food fermentation. The dried-porous-biopolymer beads are also used as a vehicle to delivery drug into the human gastrointestinal tract and control release drug the target organ (Zohar-Perez *et al*., 2004). There is, however, no report on using freeze-drying to modify the structure and properties of gelatinized-tapioca starch beads. The advantages of TSB are non-toxic in nature, very low cost (24 Baht per kg), easy to handle and commercially available as a food ingredient.

In this study, moisture content, microstructure, porosity, total bulk volume, adsorption properties (adsorption capacity and adsorption behaviour), and gel strength of FDTB were studied. The potential of the FDTB to immobilized bifidobacterial cells is also discussed.

Materials and methods

Materials

Three leading brands of commercial TSB including Golden Chef® (Oriental Food Co., LTD., Bangkok, Thailand), Special Sacoo®(Lotus Co., LTD., Chiang Mai, Thailand), and Thai world® (Thai world Import Export

Co., LTD., Bangkok, Thailand) were used for preparation of FDTB. Chemicals used in the experiment were purchased from Sigma-Aldrich (Louis, MO., USA).

Preparation of SF- and QF-FDTB

The TSB were heated in boiling water at 100°C for 15 min to completely gelatinize the beads. The beads were then cooled down immediately in deionized water at 25°C and kept at 4-5°C for 24 hours, to allow the beads to completely swell. The swollen beads were either quick freezed at -176°C, 5-10 min (QF) by liquid nitrogen or slow frozen at -20°C, for 24 hours (SF) in a cold room (Shan-Yang *et al*., 1999; Saxelin *et al*., 1999). After freezing, the beads were placed in a freeze-drying system at 25°C for 72 hours to ensure completed drying. Two different supporting materials were obtained; a) quick-freeze-dried-gelatinized tapioca starch beads (QF-FDTB) and b) slow-freeze-dried-gelatinized tapioca starch beads (SF-FDTB). The samples were kept in sealed double plastic bags for further use. The moisture content and physical evaluations included microstructure, porosity, adsorption capacity and adsorption behaviour.

Examination of SF- and QF-FDTB

Initial moisture content and total bulk volume determination

The initial moisture content of the gelatinized TSB from three commercial brands (Golden Chef®, Special Sacoo®, and Thaiworld®) were measured before freezing and after drying by hot air oven at 130 ± 3°C for 48 hours or until the weight was constant (AOAC, 1998).

The total bulk volume of 100 QF- and SF-FDTB from three commercial brands (Golden Chef®, Special Sacoo®, and Thaiworld®) were measured by 10-mL graduated cylinder. The average diameter and bulk volume were then calculated.

Porosity determination

The porosity (ε) of QF- and SF-FDTB from three commercial brands were determined following the procedure of Habib *et al*. (2002) with modification. The porosity was calculated using the following equation:

$$\varepsilon = 100\ (1 - \rho_a / \rho_t)$$

Where ρ_t isthe true density and ρ_a is the granular density. The true density(ρ_t) of the tested FDTB was determined by helium displacement (Multivolume Pycnometer 1330, Micromeretics Instrument Corp., USA). The true volume (an average of five runs) was calculated by determining the volume of helium displaced by the tested FDTB during the test. The true density was then calculated by dividing the weight of the tested FDTB by the average of the true volume.

The granular density (ρ_a) of the tested FDTB was determined using glycerol displacement in 25 mL-glass Pycnometer. The granular density was then calculated by dividing the weight of the tested FDTB by the average granular volume.

Microstructure determination

The microstructure of QF- and SF-FDTB from three commercial brands were determined by following the modified method of Habib *et al*. (2002). The beads were placed on aluminium mounts using double-sided Scotch® tape and stored overnight at 0% relative humidity in tightly sealed glass desiccators. The tested FDTB and cross- section of bead were then sputter coated with a gold-palladium mixture before examined by scanning electron microscopy (Model, JSM-5910 LV, Jeol Ltd., Japan).

Initial adsorption, adsorption capacity and adsorption behavior determination

The initial adsorption of 100-QF- and SF-FDTB from three commercial brands were determined by measurement the weight of 100-rehydrated beads stored in peptone water saline (PS: 8.5 g/L NaCl, 1.0 g/L peptone water at pH 7.0) at 4-5°C during the first minute. The initial adsorption was then calculated by dividing the weight of 100-rehydrated beads by the weight of 100-QF- or SF-FDTB.

The adsorption capacity of 100-QF- and SF-FDTB from three commercial brands were calculated by dividing the weight of completely rehydrated QF- or SF-FDTB by the dried weight of QF- or SF-FDTB.

The adsorption behavior of 100-QF- and SF-FDTB from three commercial brands were determined by monitoring the weight of 100-rehydrated QF- and SF-FDTB stored in PS at 4-5°C for 48 hours. The data were collected at

0, 1, 60, 120, 180, 240, 300, 360, 420, and 480 min, or until the weight was constant.

Gel strength determination

The gel strength of the rehydrated QF- and SF-FDTB were determined following the method of Kuo-Cheng and Jer-Ying (1997) by placing the rehydrated QF- or SF-FDTB in a semi-sphere hole of a holder. Then the rehydrated QF- or SF-FDTB was compressed to deformation by a tested needle (probe type P/2), at a constant deformation rate of 2 mm/s, employing a Texture Analyzer. Mechanical strength measurement for gelatinized beads was expressed by a critical compressive stress when abrasion of gelatinized beads occurred.

Statistical analysis

The statistical analysis was conducted using analysis of variance (SPSS ver.8, 1998). If a significant main effect was detected, the means was separated with the Duncan's multiple range test (Cochran and Cox, 1957). The predetermined acceptable level of probability was 5% ($p<0.5$) for all comparisons.

Results

The TSB was the white hard beads with the diameter ca 2.1 mm. The completely gelatinized beads were transparent and had soft texture with the diameter ca 4.2 mm. Moisture content of the gelatinized TSB was statistically significant ($p\leq0.05$) between all tested brands and the values for Golden Chef®, Special Sacoo® and Thaiworld® were 91.97 ± 0.11, 92.26 ± 0.12, and 92.59 ± 0.13%, respectively. The shape of the gelatinized beads were similar and generally spherical. Dry weight of 100 beads from two freezing methods and three commercial brands were not significant difference ($P>0.05$) with the average of 0.555 ± 0.01 g per 100 beads. QF- and SF-FDTB from three commercial brands had either spherical or elliptical shape, resulting from the compaction of gelatinized beads during freezing. After freeze-drying, the moisture content of SF- and QF-FDTB from three commercial brands were not significant difference ($P>0.05$) with the average of 4.89 ± 0.29%.

The SF-FDTB provided the porous bead with the large pore size (Fig. 1a) with the average diameter of 57.04 µm whereas the QF-FDTB provided the beads with the smaller pore size (Fig. 1b) with the average diameter of 11.18 µm for all three commercial brands. The different surface property of SF-FDTB

and QF-FDTB were detected for all three commercial brands. The SF-FDTB had a puffy surface with large open pore (Fig. 1c), whereas the QF-FDTB had smooth and some part of closed surface area (Fig. 1d). The porosity of QF- and SF-FDTB from Golden Chef®, Special Sacoo®, and Thaiworld® were calculated from the true volume and granular volume of QF- and SF-FDTB and showed the results in Table 1. The statistic analysis showed only the effect of freezing method (slow- and quick freezing) on the porosity of tested FDTB. The porosity of QF-FDTB was significantly higher than that of SF-FDTB ($p<0.05$). The porosity of QF-FDTB and SF-FDTB were 90.81 ± 0.71, and 89.23 ± 0.87%, respectively.

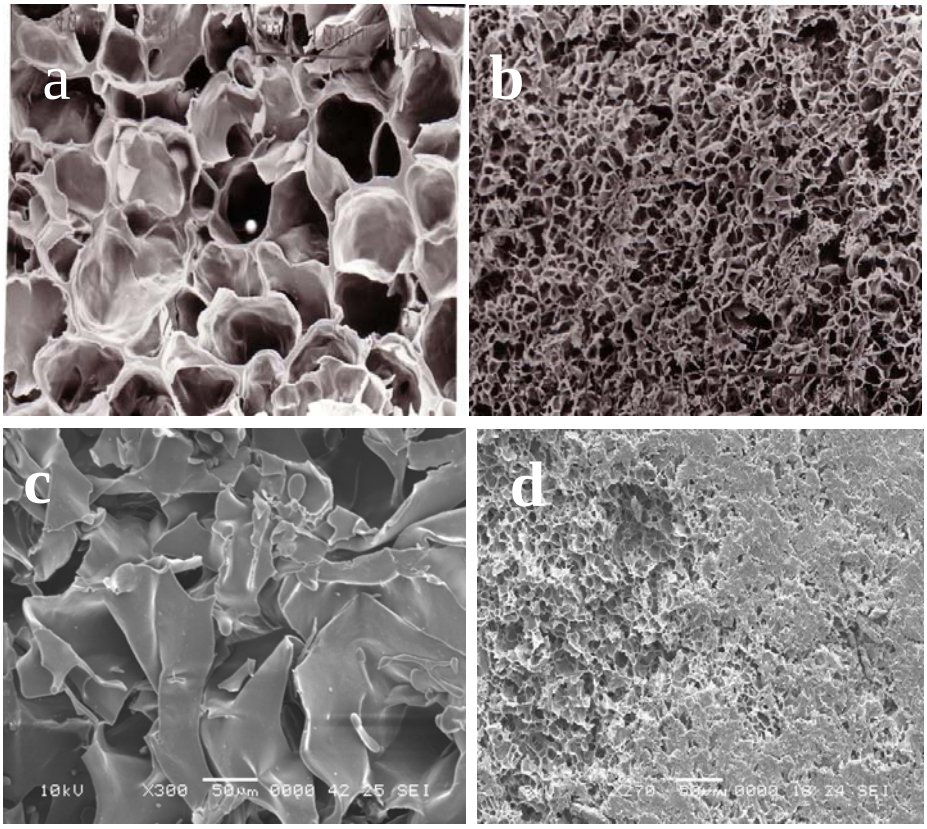

Fig. 1. Scanning electron micrograph of microstructure of SF- and QF-FDTB at magnification × 300. **(a)** Cross-section of SF-FDTB. **(b)** Cross-section of QF-FDTB. **(c)** Surface area of SF-FDTB. **(d)** Surface area of QF-FDTB

Size of QF- and SF-FDTB could be determined by using bulk volume measurement and showed the result in Fig. 2. The bulk volume of 100-QF- and SF-FDTB showed non significant difference ($P>0.05$) of the combined effect of

three commercial brands and two freezing methods but showed significant difference ($p<0.05$) of commercial brand or freezing method. The SF-FDTB had more bulk volume than the QF-FDTB ($p<0.05$) and Thaiworld® had the highest ($p<0.05$) value of bulk volume.

The statistical analysis showed the combined effect of three commercial brands and two freezing methods of FDTB on the initial adsorption of 100-QF- and SF-FDTB is significant ($p<0.05$) (Fig. 2). The SF-FDTB had higher ($p<0.05$) initial adsorption than QF-FDTB for Special Sacoo® and Thaiworld®. While Golden Chef® had similarly ($P>0.05$) result for both QF-FDTB and SF-FDTB. The adsorption capacity of QF- and SF-FDTB for all three commercial brands were not difference ($P>0.05$) with the average of 9.46 per 100 beads (Fig. 2).

Table 1. True volume, granular volume, and porosity of SF-FDTB and QF-FDTB from three commercial brand (Golden Chef®, Special Sacoo®, and Thaiworld®)

Freezing method	**Commercial Brand**	**True volume^{ns} (cm^3)**	**Granular volume (cm^3)**	**Porosity (%)**
Quick	Golden®	0.287±0.02	3.11±0.23	90.74±0.59
	Special®	0.304±0.01	3.17±0.13	90.41±0.33
	Thaiworld®	0.295±0.004	3.34±0.06	91.28±0.17
Slow	*Golden®*	0.302±0.02	2.92±0.11	89.72±0.22
	Special®	0.304±0.03	2.71±0.34	88.93±0.49
	Thaiworld®	0.358±0.01	3.32±0.23	89.05±0.74

Three commercial brands and two freezing methods of FDTB were tested for adsorption behavior in PS at 4-5°C for 24 hours. Similarly adsorption patterns of SF-FDTB and QF-FDTB from Golden Chef®, Special Sacoo®, and Thaiworld® were noticed (Fig. 3). The adsorption rate of SF-FDTB was very fast in the first 50 min and the adsorption was maximal at 240 min. The maximum adsorption of QF-FDTB was reached at 360 min.

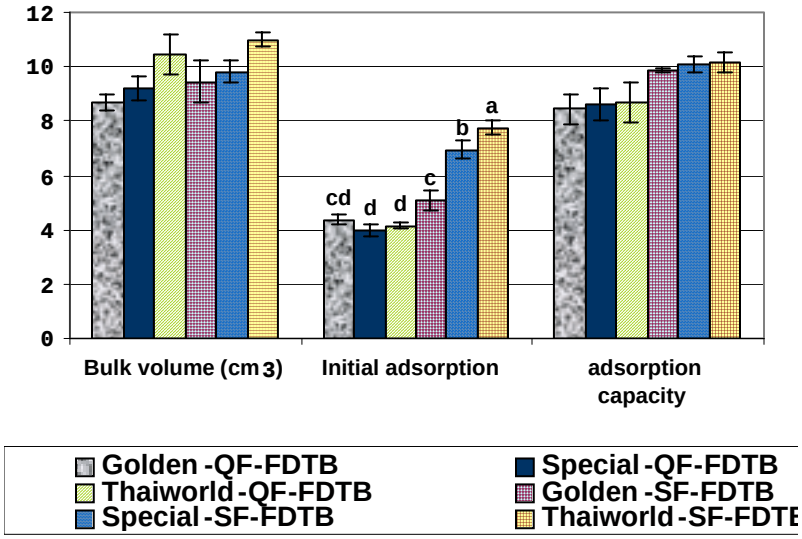

Fig. 2 Bulk volume, initial adsorption, and adsorption capacity of SF-FDTB and QF-FDTB from three commercial brand (Golden Chef®, Special Sacoo®, and Thaiworld®). Each value in the figure represents the mean from three replicates. Results of initial adsorption with different letter are significant difference ($P<0.05$ by Duncan's multiple range test). Standard error bars are included.

The gel strength of the three commercial brands produced by two freezing methods were significant difference ($p <_ 0.05$) (Fig. 4). The firm texture was detected from the Golden Chef®FDTB processed by quick freezing and slow freezing. The gel strength of Golden Chef®QF-FDTB and SF-FDTB were 27.35 $\pm$ 1.33 and 22.18 $\pm$ 0.14 g, respectively. The lowest gel strength of 7.2 $\pm$ 0.39 g was detected from the Thaiworld®SF-FDTB.

Discussion

Slow freezing and quick-freezing contributed to the distinguished the properties of FDTB. The puffiness of the outer surface area, expanded dimension, and large pore size were detected in SF-FDTB. In contrast, QF-FDTB showed the smooth outer surface area and very small pore size (Fig. 1b, d). A possible reason for this observation is that during slow freezing, ice crystals could grow in both intragranular and intergranular spaces. Ice crystals have a lower water vapour pressure than regions within the granular, and water therefore moves from the granular to the growing crystals, resulting in

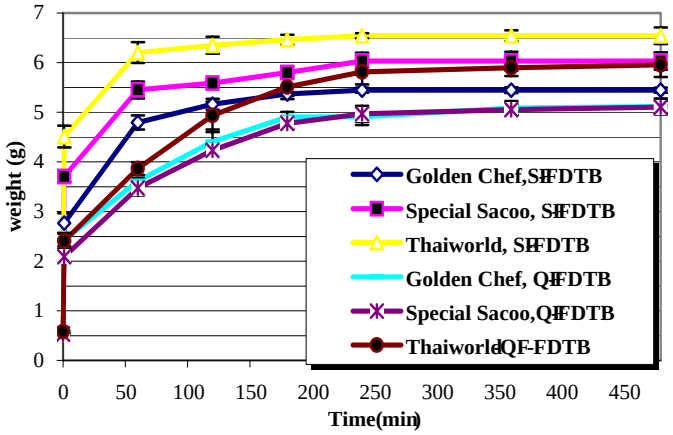

Fig. 3 Adsorption behavior of SF-FDTB and QF-FDTB from three commercial brand (Golden Chef®, Special Sacoo®, and Thaiworld®). Each value in the figure represents the mean from three replicates. Standard error bars are included.

deforming of the structure of the beads. In addition, the volume of ice could expand the dimension of soft gel beads after freezing. Quick freezing, causes the granular surface to form a crust and prevents further expansion and smaller ice crystals form within intragranular spaces that reduce damage to the structure of the bead. During freeze-drying process, the ice sublimes directly to vapour without melting (Fellows, 2000). When the beads were completely dried, the porous beads were obtained and pore size of the FDTB depended on the size of ice crystal. The large pore size of SF-FDTB created spongy texture that allows rapid rehydration and had higher initial adsorption (Figs 2, 3), but decreased the gel strength of the rehydrated beads (Fig. 4). The porosity of QF-FDTB was higher than that of SF-FDTB and the results agreed with the experiment of Shan-Yang *et al*. (1999). Structure modification of gel beads by using freeze-drying in order to produce the porous structure was also reported by Tal *et al*. (1997) and Whitehead *et al*. (2000) who produced the porous alginate beads. Fwu-Long *et al*. (2002) produced freeze-dried chitosan beads.

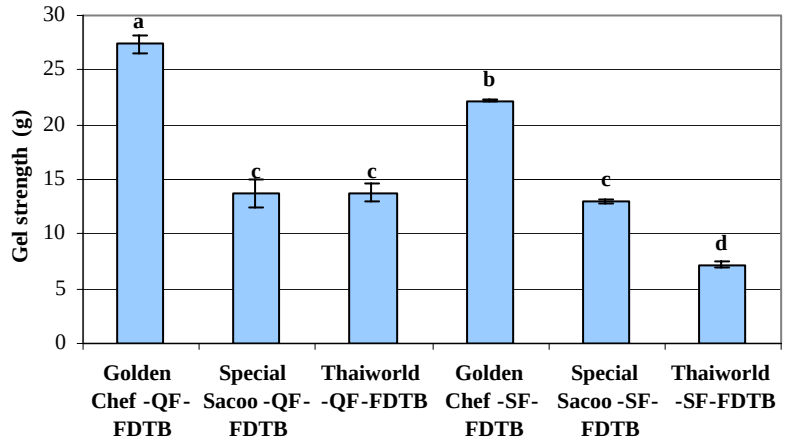

Fig. 4. Gel strength of SF-FDTB and QF-FDTB from three commercial brand (Golden Chef®, Special Sacoo®, and Thaiworld®). Each value in the figure represents the mean from three replicates. Results with different letter are significant difference ($P<0.05$ by Duncan's multiple range test). Standard error bars are included.

The mechanisms involved the water adsorption behaviors of QF- and SF-FDTB, which may be the hydrophilic property and water capillary attraction. Water may bind to the starch granule of QF- and SF-FDTB via hydrogen bonding which cause the beads to hold water inside the bead. The maximum adsorption of SF-FDTB occurred faster than that of QF-FDTB. The reason may be due to the open surface characteristic and larger pore size of the SF-FDTB that allows water to penetrate more easily. The QF-FDTB had some parts with a closed surface area and smaller pore size that may allow to water gradually penetrate into the beads. The maximum adsorption of QF- and SF-FDTB reached when the starch granules were fully hydrated. Once hydration occurred, hydrogen bonding between the amylose and amylopectin maintain the integrity of the granules and associate to form the matrix structure (Fennema, 1996). The matrix structure may allow the beads to adsorb more water. In this study, the adsorption capacity of FDTB was not correlated to the specific surface area of the beads (data not shown) but was affected by the pore size and adsorption behavior of the beads, which was modified by freezing method.

Rehydrated SF- and QF-FDTB has large dimension (diam. ca 4.2 mm) with soft and smooth texture which is easy to consume. People in many countries are familiar to TSB and use gelatinized TSB as the habitat food and as source of carbohydrate. SF- and QF-FDTB have the benefits of being nontoxic to the cells being immobilized, and it is an accepted food ingredient.

Accordingly, TSB could be the ideal carrier for immobilization. Results from this study could provide fundamental information for the further study with immobilization of probiotic cell in FDTB. Though SF-FDTB showed higher adsorption property than QF-FDTB, however, the capacity to load bifidobacterial cells into the beads should be studied and compared between SF- and QF-FDTB from three commercial brands (Golden Chef®, Special Sacoo®, and Thaiworld®). The dimension of bifidobacterial cells was 0.5-1.5 × 1.5-8 µm (Tamime and Robinson, 2000). Comparison of the dimension of bifidobacterial cells and the pore size of QF-FDTB (diam. ca 11.18 µm) and SF-FDTB (diam. ca 57.4 µm) indicates that the bifidobacterial cells may easily pass through the porous structure of QF- and SF-FDTB. However, bifidobacteria are non-motile (Ballongue, 1998), then it is necessary to use the adsorption property of QF- and SF-FDTB to adsorb bifidobacterial cells into the beads.

Conclusions

The properties of gelatinized TSB including, microstructure, porosity and adsorption property could be modified by freezing method with different temperatures. Bacterial immobilization in FDTB could be a new and yet simple technique that enhances delivery of viable probiotics culture to the intestinal tract.

Acknowledgements

The authors thank the Thailand Office of the University Affairs in providing a grant to conduct this study under Doctoral in Agro-Industry Program.

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(Received 29 September 2005; accepted 29 November 2005)