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Enzymatic Modification of Sea Buckthorn Dietary Fiber by Xylanase from *Streptomyces rameus* L2001: Characterization of its Physicochemical Properties and Physiological Effects on *Bifidobacterium*

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Abstract

Xylanase from *Streptomyces rameus* L2001 was used to modify sea buckthorn dietary fiber to enhance the amount of soluble fiber in this study. We then investigated the physicochemical and physiological properties of the resulting dietary fiber. Conventional single factor methods and response surface methods were used to optimize the conditions and the outcomes were as follows: hydrolysis time, 5 h; enzyme content, 46 U/g; temperature, 55.7°C. Under these conditions, the yield of modified soluble dietary fiber was 42.84 mg/g, as compared with 14.04 mg/g for unmodified fiber. We also conducted studies to determine the properties of modified sea buckthorn dietary fiber, including water holding capacity, oil binding capacity and swelling capacity, which were 110.7%, 106.9% and 116.5%, respectively, relative to unmodified fiber. We next cultivated *Bifidobacterium* with the soluble dietary fiber (derived from modified sea buckthorn) and compared growth with that of bacteria maintained on glucose. Supplying *Bifidobacterium longum* and *B. infantis* with the soluble component aided their proliferation, particularly of *B. longum.* This is the first report on sea buckthorn dietary fiber and its modification with xylanase.

Keywords: Sea Buckthorn Dietary Fiber, Xylanase, Modification, Physicochemical and Physiological Properties

1. Introduction

Sea buckthorn is a new multifunctional food with significant agricultural, ecological, nutritional, medical and ornamental value. (Ruan and Li, 2005) Because of its commercial and nutritional value, sea buckthorn has been the focus of much research to determine its basic physiochemical characteristics, as well as the physiological properties of different components and compounds, including seed oil, flavones, tocopherol and phenolic acid, and the putative associations between these compounds and human health. Larmo *et al*. (2009) researched the effects of sea buckthorn berries on circulating concentrations of cholesterol, triacylglycerols and flavones in healthy adults. They found that sea buckthorn berries have benefits on human health in terms of cardiovascular disease risk by reducing C-reactive protein concentration. A similar conclusion was reached by Xu *et al*. (2011). Other studies have shown that sea buckthorn berries are a source of phenols and proanthocyanidins that may help suppress the growth of human colon and liver cancer cells (Grey *et al*., 2010). Moreover, because of the different phytonutrients and bioactive substances present in sea buckthorn, it has beneficial effects on wound healing (Gupta *et al*., 2006), immune function, oxidative stress by stabilizing membrane structure in animals and increasing the activities of essential enzymes (Yang and Kallio, 2002).

Research into sea buckhorn has also focused on the identification and extraction of its components. High-performance liquid chromatographic fingerprinting has been used to identify the different origins of sea buckthorn berries (Chen *et al*., 2007). However, no systemic research studies of sea buckthorn dietary fiber have been performed to date. Several observational studies have shown that dietary fiber intake is associated with a number of health benefits, and that these effects are dependent on the composition (i.e., soluble and insoluble components) and physicochemical properties of the dietary fiber (Cornfine

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et al., 2010). It is now well established that certain sources of dietary fiber, such as psillium, pectin and oats, can lower serum cholesterol and blood sugar concentrations, independently of the fat or carbohydrate content of the diet (Hu *et al*., 2008), as well as decrease the prevalence of cancer, particularly colon and breast cancer, improve body weight management and may indirectly reduce the risk of heart disease (Kendall *et al*., 2010). Some studies have indicated that the quantity and activity of the microflora in the gut are related to the relative utilization of fermentable dietary fiber (Guillon and Champ, 2000). As described above, research into sea buckthorn fiber is important, not only in terms of empirical evidence, but also considering its potential effects on human health. Further research on sea buckthorn fibers is important to determine the relationship between the components and properties of dietary fiber with health. Such research will also increase the availability of fiber-rich food to provide more nutritional materials for use in daily life.

The purpose of this study was to modify sea buckthorn dietary fiber with xylanase obtained from *Streptomyces rameus* L2001, and evaluate the physicochemical properties of modified dietary fiber (MDF), total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Outcomes of interest included the water holding capacity (WHC), oil binding capacity (OBC) and swelling capacity (SC). The optimized conditions were determined using the single factor method in addition to response surface methodology with a three-level, three-variable central composite rotational design.

Several small-scale clinical studies with various fermentable dietary fibers have shown significant, but small, clinical benefits of these products either alone or in combination with probiotics on a number of intestinal diseases and disorders (Bittner *et al*., 2007). Based on these properties of dietary fiber, bacteria capable of fermenting carbohydrates (*B. longum* and *B. infants*) were cultured with soluble dietary fiber (derived from modified sea buckthorn) to investigate its effects on bacterial growth.

2. Materials and Methods

2.1. Xylanase Purification and Assay

Streptomyces rameus L2001, isolated from soil samples (Tianshan, Xinjiang), was used in this study, and the purified process was according to Xiuting Li et al. (2010). Briefly, the strain was fermented in culture broth for 5 days at 40℃, and the production of the xylanase was nearly 2000U/mL. After centrifuging (8000×g for 10 min at 4ºC) and ammonium sulfate precipitation (40-60%) , the dialyzed enzyme solution was applied to a DEAE-52 column (1.0cm×10cm) and CM Sepharose Fast Flow column (1.0cm×10cm), which were pre-equilibrated with 20mM Tris-HCl buffer (pH=7.0) and 20mM acetate buffer (pH=5.3) , respectively. The purpose protien were eluted with 0-0.05M NaCl gradient at a flow rate of 1.0 mL/min. All purification procedures above were performed at 4 ºC unless stated otherwise. Ultimately, the purified xylanase was checked by SDS-PAGE to determine the purity and protein molecular.

The reducing sugar produced in this experiment was assayed by the dinitrosalicylic (DNS) acid method with xylose as the standard, and the one unit of xylanase activity was defined as the amount of enzyme that catalyzes the release of 1μmol of xylose equivalent in one minute under the assay conditions.

2.2. Preparation of Sea Buckthorn Dietary Fiber

Sea buckthorn was provided by Qinghai kangpu biological technology Co., Ltd (Qinghai, PR China), milled through a 300-μm mesh, and heated at 65°C in a air-drying oven (Modle BA0-35A, Shidokai Shanghai Equipment Co., Ltd., Shanghai, China). A sample of sea buckthorn was then immersed in a 10-fold volume of acetate buffer (pH 5.8) and heated to 70°C for 30 min with continuous stirring. In order to gain more soluble fiber, 2 mL of neutral protease (Novozymes Biological Technology Co., Ltd., Beijing, China) was added to the mixture to remove the protein. A conical flask (500 mL) containing the sea buckthorn suspension was heated to 45°C in a water bath (Model SHY-2, Jinchengguosheng Instrument Plant, Jiangsu, China) for 2 h with agitation at 180 rpm. After enzyme hydrolysis, 4 volumes of 95% ethanol were added to precipitate the polysaccharides, and incubated for 4 h at 4°C. The precipitate was collected by centrifugation at 5000 ×*g* for 15 min, followed by vacuum drying to obtain the dietary fiber (DF) used in this study.

2.3. Composition and Physical Properties of Dietary Fiber

The TDF, IDF and SDF in dietary fiber and XMF were determined using the AOAC method. WHC, OBC and SC were determined as previously described (Sangnark and Noomhorm, 2003; Escalada-Pla, 2007).

2.4. Processing of Sea Buckthorn Dietary Fiber by Xylanase from Streptomyces rameus L2001

Hydrolysis of sea buckthorn dietary fiber (5g) was performed in a 50-mL conical flask containing acetate buffer (pH 5.8). The initial temperature and fluid material ratio were 50°C and 10. Based on the pilot studies performed to determine the characteristics of xylanase hydrolysis, the following experimental conditions were used: hydrolysis time, 1–6 h; enzyme dose, 15–65 U/g, temperature, 45–65°C.

2.5. Experimental design and statistical analysis

A three-level, three-variable central composite rotatable design was applied using Design-Expert software version 8.0.1 (State-Ease, Inc., Minneapolis, MN, USA) to determine the optimal enzymatic conditions. Based on our single-factor experiment, the three variables were hydrolysis time, 2–4 h; enzyme dose, 35–55 U/g, and hydrolysis temperature, 50–60°C. The response variable was SDF content (mg/g). Each variable was coded at three levels (–1, 0 and 1), as shown in Table 1.

Table 1.Variables and their levels used in a central composite rotatable design for optimization of xylanase hydrolysis conditions

Variable	Coded level		
	–1	0	1
Hydrolysis time (h)	2	3	4
Enzyme dosage (U/g)	35	45	55
Hydrolysis temperature (°C)	50	55	60

Taking into account the main, quadratic and interaction effects, the quadratic response surface analysis was based on the multiple linear regressions presented in Eq. (1). As the three parameters were varied, 10 α-coefficients were to be estimated, corresponding to the three main effects, three quadratic effects, three interactions and one constant.

$$Y=\alpha_0+\sum_{i=1}^{3}\alpha_i x_i+\sum_{i=1}^{3}\alpha_{ii}x_i^{\,2}+\sum_{i=1}^{2}\sum_{j=i+1}^{3}\alpha_{ij}x_i x_j+e \quad (1)$$

The response function was Y=mg/g of modified soluble sea buckthorn dietary fiber.

2.6. Bifidobacterium *Species and Proliferation Assay*

B. longum and *B. infantis* were provided by the Chinese Academy of Agricultural Sciences (Beijing, China). Both strains were activated using basal medium (g/L) containing 10 g tryptone, 10 g yeast extract, 10 g beef extract, 2 g cysteine hydrochloride, 20 g glucose, 0.25 g K_2HPO_4, 0.58 g $MgSO_4{\cdot}7H_2O$, 0.25 g $MnSO_4$, 5 g sodium acetate anhydrous (pH 6.8). The bacterial strains all grew statically in this medium for 24 h in an anaerobic chamber (Model 1029, Thermo Fisher Scientific, Waltham, MA, USA) at 37°C.

To assess the effect on bacterial proliferation, sea buckthorn SDF (derived from MDF) was added to the culture medium instead of glucose (20 g/L, enriched medium), while the other nutritional components were retained. The growth of *B. longum* and *B. infantis* were assayed by measuring absorbance at 620 nm using a spectrophotometer (Shanghai Lingguang Technology Co., Ltd., Shanghai, China). Briefly, two culture flasks were prepared; one contained 50 mL of basal medium while the other contained 50 mL of enriched medium. Then, 4% activated bacterial suspension was added to each flask, followed by culture for 48 h at 37°C in an anaerobic chamber. Bacterial growth was assayed every 6 h and growth curves were plotted. Proliferation studies were performed at least in duplicate and the mean was calculated.

3. Results and Discussion

3.1. TDF, IDF and SDF Content of Sea Buckthorn

The composition of sea buckthorn dietary fiber was determined before modification. As shown in Table 2, the amounts of TDF, SDF and IDF were 68.03±0.12%, 2.88±0.21% and 65.15±0.33%, respectively. These data indicate that sea buckthorn has abundant fibers. Compare with other fruits, the TDF and IDF contents were 0.3% and 0.6% in watermelon, and 9.1% and 10.9% in sapota, respectively. Meanwhile, the SDF content was 0.3% in watermelon and 2.4% in fig (Ramulu and Rao, 2003). Almost 25 different fruits were assayed, and the values of TDF, IDF and SDF were consistently lower than those in sea buckthorn dietary fiber in this study. These results indicate that sea buckthorn contains much higher dietary fiber than other fruits and it may become a new and good resource of fibers for daily life, even for food by-prodcut or industry as other plants and fruits.

Table 2. Composition of sea buckthorn

Composition	Content
SDF	2.88±0.21%
IDF	65.15±0.33%
TDF	68.03±0.12%

The insoluble and soluble properties of dietary fiber are related to differences in their physiological functions and effects (Jimenez-Escrig and Sanchez-Muniz, 2000). Insoluble fibers are characterized by their porosity, low density, and their abilities to increase fecal bulk and decrease intestinal transit. In contrast, soluble fibers are characterized by their capacity to increase viscosity, and to reduce the glycemic response and plasma cholesterol levels (Abdul-Hamid and Luan, 2000). Moreover, foods high in SDF, such as fenugreek (Al-Habori and Raman, 1998), guar gum, oat and psyllium have hypoglycemic and hypocholesterolemic effects in experimental animals and in humans (Bittner *et al*., 2007). In this paper, we used xylanase to modify sea buckthorn dietary fiber to enhance the SDF content and investigated the physicochemical properties of modified sea buckthorn fiber to assess its function and biological value.

3.2. Effects of xylanase hydrolysis time on SDF content

Figure 1 shows the effect of hydrolysis time on the SDF content of sea buckthorn dietary fiber. Between 1 and 3 h, the SDF increased, reaching a peak of 36.16 mg/g. Meanwhile, extending the reaction time over 3 h decreased SDF content.

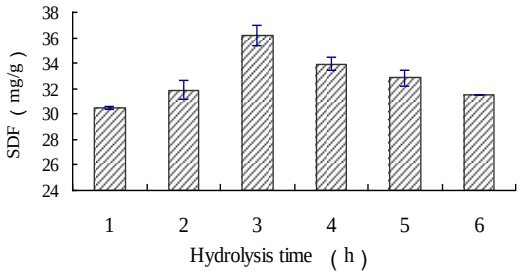

Figure 1. Effects of hydrolysis time on the SDF content of sea buckthorn (mg/g). Reaction conditions: 50 mL acetic acid (pH 5.3), 40 U/g enzyme, temperature 50°C, and incubation for 1–6 h.

The substrate and enzyme concentrations have marked effects on the kinetics of enzymatic hydrolysis (Yoon *et*

al., 2005). During the early phase of the reaction, a high substrate concentration and low levels of polysaccharide had a weak inhibitory effect on xylanase activity, allowing the hydrolysis reaction to occur rapidly. However, as the reaction proceeding, the polysaccharide concentration increased and competitively inhibited the enzymatic reaction, reducing its speed.

3.3. Effects of Enzyme Dose on SDF Content

Figure 2 shows the effects of various doses of xylanase on the yield of SDF. And the greatest yield (37.25 mg/g) was at an enzyme dose of 45 U/g. This experiment showed that increasing the doses of xylanase in a suitable range, increasing the yield of SDF by converting insoluble dietary fiber into polysaccharides, oligosaccharides or monosaccharides.

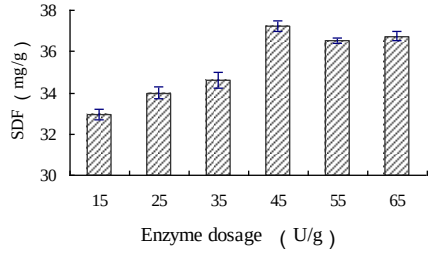

Figure 2. Effects of enzyme dose on the SDF content of sea buckthorn (mg/g). Reaction conditions: 50 mL acetic acid (pH 5.3), hydrolysis time 3 h, temperature 50°C, enzyme dose 15–65 U/g.

3.4. Effects of Hydrolysis Temperature on SDF Content

Figure 3 shows the effects of hydrolysis temperature on SDF content. SDF content increased with increasing temperature until 55°C, and then decreased at higher temperature.

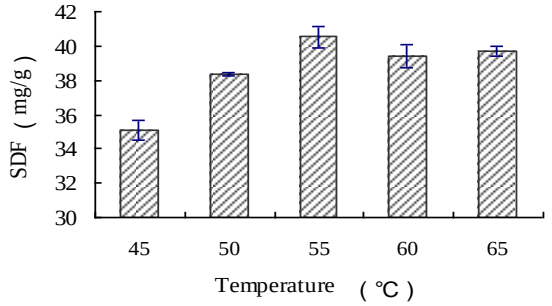

Figure 3. Effects of different hydrolysis temperatures on SDF content of sea buckthorn (mg/g). Reaction conditions: 50 mL acetic acid (pH 5.3), hydrolysis time 3 h, enzyme dose 45 U/g, and hydrolysis temperature 45–65°C**.**

3.5. Optimization of the Xylanase Modification Conditions

According to the different hydrolysis conditions that affected the content of SDF, as shown in Figs. 1–3, we selected a hydrolysis time of 3 h, enzyme dose of 40 U/g and hydrolysis temperature of 55°C as the central conditions of the response surface analysis to optimize the production of SDF.

The coefficients derived from analysis of variance are shown in Table 3.

Table 3. Analysis of variance for the response surface quadratic model

Source	df	Sum of Squares	Mean Square	F	Pr>F	
X_1	1	4.82	4.82	3.78	0.0928	
X_2	1	7.30	7.30	5.73	0.0479	*
X_3	1	0.86	0.86	0.68	0.4372	
X_1X_2	1	0.099	0.099	0.078	0.7882	
X_1X_3	1	0.16	0.16	0.13	0.7335	
X_2X_3	1	0.027	0.027	0.021	0.8879	
X_1^2	1	0.049	0.049	0.039	0.8499	
X_2^2	1	54.10	54.10	42.47	0.0003	*
X_3^2	1	29.28	29.28	22.99	0.0020	*
Lack of fit	3	8.43	2.81	23.20	0.0054	*
Model	9	101.32	11.26	8.84	0.0045	*
Pure error	4	0.48	0.12			

X_1: Hydrolysis time (h); X_2: enzyme dose (U/g); X_3: Hydrolysis temperature (°C); *: significant; [a] Coefficient of determination (R^2) = 0.9191; [b]CV% = 3.00%.

The linear coefficients of enzyme dose and the two quadratic terms for enzyme dose and hydrolysis temperature were statistically significant (P<0.05). While, interactions between the three independent variables was not statistically significance (P>0.05), suggesting little interaction between these parameters. The total coefficient of determination (R^2) and the Model index was 0.9191 and 0.0045 (P<0.005), respectively, indicaed a reasonable fit of the model to the experimental data. And the predicted yield of modified SDF was calculated using the regression model and were compared with the experimental values in Figure 4.

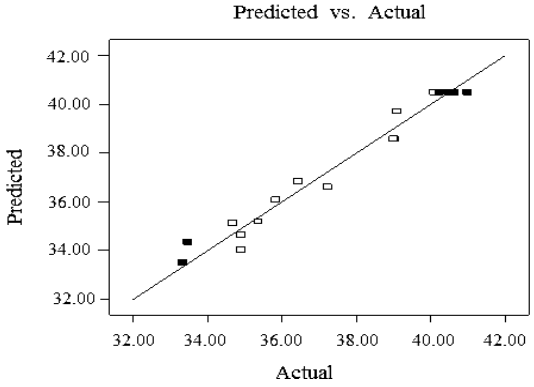

Figure 4. Comparison between the predicted and actual yields of modified SDF.

$$Y=40.48+0.78X_1+0.96X_2+0.33X_3-0.16X_1X_2+0.\mathbf{2X_1X_3}+0.082X_2X_3+0.11X_1^2-3.58X_2^2-2.64X_3^2 \quad (2)$$

To determine the optimum conditions for greatest SDF yield, three-dimensional response surface plots were constructed based on Eq. (2).

Figure 5 shows the linear effects of hydrolysis time and quadratic effects of enzyme dose on SDF content. SDF yield reached at a maximum value near the central condition, and with the hydrolysis proceeding, the production was increased at any enzyme dosage.

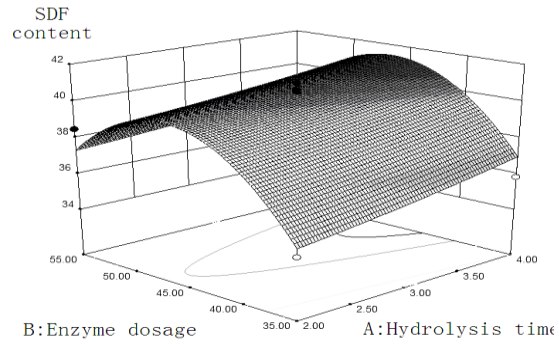

Figure 5. Response surface plot showing the effects of hydrolysis time (h) and enzyme dose (U/g) on SDF content. Hydrolysis temperature (55°C), 50 mL acetic acid and pH (5.3) were kept constant

Figure 6 shows the quadratic effects of hydrolysis temperature and linear effects of hydrolysis time on SDF content; it was similar to Fig. 5.

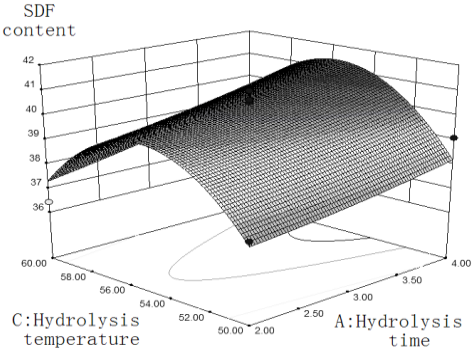

Figure 6. Response surface plot showing the effects of hydrolysis time (h) and hydrolysis temperature (°C) on SDF content. Enzyme dose (45 U/g), 50 mL acetic acid and pH (5.3) were kept constant.

The contour plot shows both quadratic effects of hydrolysis temperature and enzyme dose on SDF content in Figure 7.

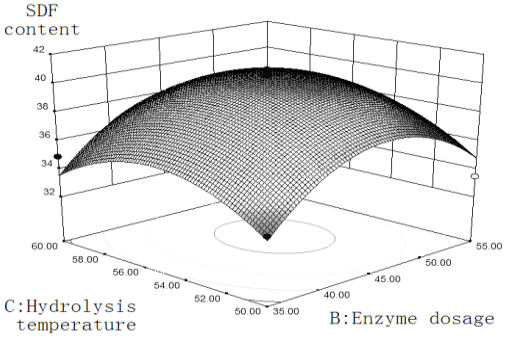

Figure 7. Response surface plot showing the effects of enzyme dose (U/g) and hydrolysis temperature (°C) on SDF content. Hydrolysis time (3h), 50 mL acetic acid and pH (5.3) were kept constant.

This figure shows that the response surface had a maximum point, when the optimum hydrolysis temperature and enzyme dose were 55°C and 45 U/g, the maximum SDF content was 40.53 mg/g.

The optimal conditions derived from the model were as follows: hydrolysis time, 5 h; enzyme dose, 46 U/g; hydrolysis temperature, 55.7°C. After xylanase modification, the amount of soluble dietary fiber increased from 2.88% to 4.28%. Although the enzymatic method to modify the sea buckthorn dietary fiber is not desirable than we expectation, it is also meaningful to reveal the crude xylanase has ability to modify the cereal fiber to gain solube fibers. Meyera *et al*. (2009) modified potato pulp with an enzyme derived from fungi, and found that this did not alter the SDF yield or the relative amount of soluble fiber in the total solubilized dry matter. Meanwhile, other studies focus on digestibility and short chain fatty acid production, rather than SDF concentration (Carneiro *et al*., 2008). In recent years, more researches have been conducted on the physical modification of dietary fibers. For example, Repo-Carrasco-Valencia *et al*. (2009) modified *Amaranthus caudatus* fiber by extrusion, which increased the SDF content from 2.45% to 3.06% in one variety, whereas a slight decrease from 1.65% to 1.46% was noted in another variety. Because the food sensorial properties of food may be affected by chemical treatments and mechanical procedures are associated with low yield, the use of enzymes for modifying cereal-based foods may offer an approach to overcome such limitations (Napolitano *et al*., 2009).

3.6. Physical Properties of Sea Buckthorn IDF, SDF, TDF and MDF

Figure 8 shows the value of WHC of the SDF and IDF. Interestingly, the OBC and SC tended to be greater for SDF than for IDF. The SC and WHC are indices of fiber hydration and provide useful information for fiber-supplemented foods. These properties will also help us to predict the behavior of fiber in foods or during gut transit (Xu *et al*., 2011).

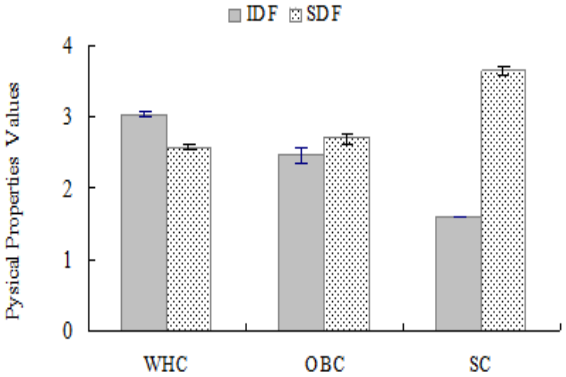

Figure 8. Water holding capacity (WHC), oil binding capacity (OBC) and swelling capacity (SC) of insoluble (IDF) and soluble (SDF) dietary fiber.

As shown in Figure 9 the WHC, OBC and SC of TDF were 3.31±0.034, 2.86±0.084 and 1.70±0.1, respectively. Compared with other cereal and fruits, the SC was much lower in TDF than in apple fiber (3.8) and citrus fiber (8.6). While the WHC was similar to that of wheat bran (3.0), maize bran (2.4) and resistant starch (3.1), as reported by Guillon and Champ (2000). Finally, the OBC

was favorable compared with that of dry okra (3.18) (Schneeman, 1999).

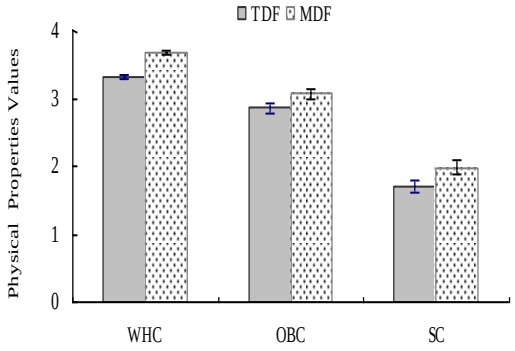

Figure 9. Water holding capacity (WHC), oil binding capacity (OBC) and swelling capacity (SC) of total (TDF) and modified (MDF) dietary fiber.

In a comparison of these parameters between TDF and MDF, the values tended to be higher for MDF than for TDF. This indicates that xylanase modification improved the physical characteristics of sea buckthorn, and these improvements were likely due to the modification of the physical structure of the dietary fiber (McCleary *et al*., 2001). Enzymatic modification is useful to convert the insoluble fiber into soluble oligosaccharides, and alter the structure of dietary fiber, including particle size and surface area, and thus influence the hydration properties. Although the enzymatic degradation role of xylanase is limited and the mechanisms involved in the changes about the physical properties of MDF are still not fully understood, it seems that partial degradation of the dietary fiber by xylanase is likely to lose the structure of dietary fiber and enable swelling. Consequently, oil and water molecules can bind more easily with the individual sea buckthorn fibers particle.

Processes, such as grinding, drying, heating or extrusion cooking, if they modify the physical properties of the fiber matrix, also affect the hydration properties (i.e., WHC and SC) (Renard *et al*., 1994). Raghavendra *et al*. (2006) reported that decreasing particle size from 1127 to 550 μm using a screw press increased the hydration properties. Physical processes have some disadvantages, including wastage of material and the cost due to use more resources and power to obtain the desired products. Accordingly, finding an appropriate method to modify the dietary fiber is becoming increasingly important and many researchers are now focusing on enzyme to modify dietary fiber because its process and approach is more moderate than physical methods, and greater yields can be obtained.

3.7. Effect Of Dietary Fiber On Proliferation Of Bifidobacteria

Bifidobacteria are gram-positive, saccharolytic anaerobic bacteria, and comprise up to 25% of the cultivable gut microflora (Duncan *et al*., 2007), they obtain carbon and energy by fermenting the host's dietary carbohydrates. Furthermore, the different species of *Bifidobacteria* differ in their fermentation profiles when cultured with different fermentable carbohydrates (Yuan *et al*., 2005), and IDF is generally more resistant to colonic fermentation than SDF (Jenkins and Kendall, 2000). Among the *Bifidobacteria*, *B. longum* and *B. infantis* have been studied for their effects on human health and incorporated into dairy products and therapeutic preparations (Biavati and Mattarelli, 2001). Therefore, we used SDF derived from MDF as the carbon source and compared fermentation profiles and growth with both of *Bifidobacteria* cultured with glucose.

Figure10 shows the effects of SDF and glucose as the sole carbon source on the growth of *B. longum*. By 6 h, bacteria in both groups had started to proliferate, with a more pronounced effect in bacteria grown in SDF-enriched medium. By 18 h, the optical density (OD) values started to reach a plateau in the SDF group, indicating that the growth of *B. longum* had started to stabilize. In contrast, the growth of *B. longum* cultured in glucose was much slower over 0–24 h, and the plateau was evident at 24 h. Based on these data, the use of SDF as the fermentation carbohydrate source achieves more rapid growth of *B. longum* than glucose.

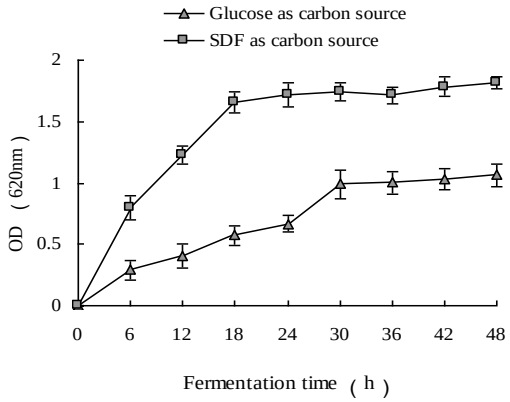

Figure 10. Comparison of the effects of SDF and glucose on the growth of Bifidobacterium longum. Experimental conditions: medium 50 ml (2% modified SDF and glucose in enriched and basal medium, respectively), fermentation time 48 h, temperature 37°C, anaerobic fermentation.

A similar study was performed by Arrigoni *et al*. (2002), who used a wheat germ preparation to cultivate *Bifidobacteria* in vitro. They found that the wheat germ preparation increased the proportion of *Bifidobacteria* from 15% to 24% of all bacteria detected in the faecal matter. Although their results and methodology differ somewhat from our own, the results of both studies indicate that dietary fiber is beneficial for microbial growth and symbiosis in the human intestine.

We next tested the effects of SDF on the growth of *B. infantis*. As shown in Figure 11, the growth curves of *B. infantis* was similar to those of *B. longum*. As before, the OD value was consistently higher for bacteria grown in the SDF-enriched medium than those grown in the basal glucose medium, which was apparent by 6 h. Bacterial growth in the SDF-enriched medium continued to increase over time, reaching the plateau phase at 36 h. In comparison, bacteria grown in the basal glucose medium reached the plateau phase at 42 h. This indicates that *B. infantis* and could catabolize SDF as the carbohydrate source more efficiently than glucose.

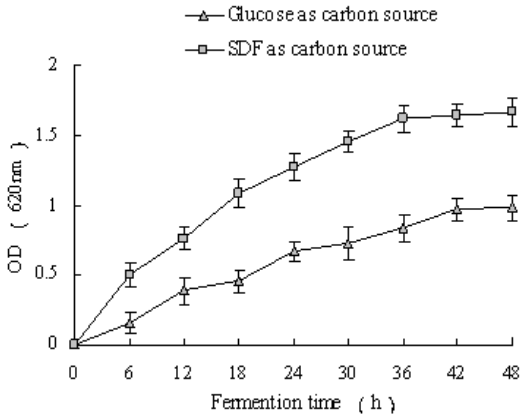

Figure 11. Comparison of the effects of SDF and glucose on the growth of *Bifidobacterium infantis*. Experimental conditions: medium 50 ml (2% modified SDF and glucose in enriched and basal medium, respectively), fermentation time 48 h, temperature 37°C, anaerobic fermentation.

In this study, both *B. longum* and *B. infantis* showed enhanced growth in SDF-enriched medium than in basal glucose medium. These results demonstrate that the dietary fiber has marked effects on proliferation of bacterial communities. Another study has shown that the type of fiber is a major factor that affects the bacterial community, and that administration of dietary fiber may promote animal or human health aiding bacterial growth and fermentation to short chain fatty acids (Amado and Arrigoni, 1992). In addition, it seems that the chemical composition of dietary fiber, particularly SDF, may affect bacterial fermentation. This concept is supported by a study performed by Lebet *et al*. (1998) who found that apple pomace and celery cell wall pectic substances were easily metabolized by the microflora and that oat bran fermentation was characterized by rapid degradation of mixed-linked β-glucans and starch. It was therefore concluded that soluble polysaccharides are more beneficial for bacterial fermentation and the amount of SDF seems to be the most important determinant for the fermentability of fiber-rich substrates because this fraction is easily and completely fermentable (Cummings and Macfarlane, 1991) and its monomer composition dictates the short-chain fatty acid profiles. Accordingly, we conducted enzymatic modification increased the content of SDF and altered the structure and physicochemical properties of sea buckthorn dietary fiber.

The growth of a microorganism on a particular oligosaccharide may be strain specific because of differences in the transport systems of oligosaccharides (Holt *et al*., 2005). Figure 12 compares the effects of sea buckthorn SDF on the growth of *B. infantis* and *B. longum*. By comparing the OD values, *B. longum* seems to grow more efficiently on sea buckthorn SDF than *B. infantis*. Wang *et al*. (2010) compared the utilization of xylooligosaccharides (XOS) with that of wheat bran dietary fiber by *B. adolescentis*, *B. longum*, *B. bifidum* and *B. breve*. They found that *B. adolescentis* displayed the highest growth rate on XOS, followed by *B. longum*. Based on these observations, it is apparent that different species of bacteria metabolize dietary fiber and carbohydrates differently.

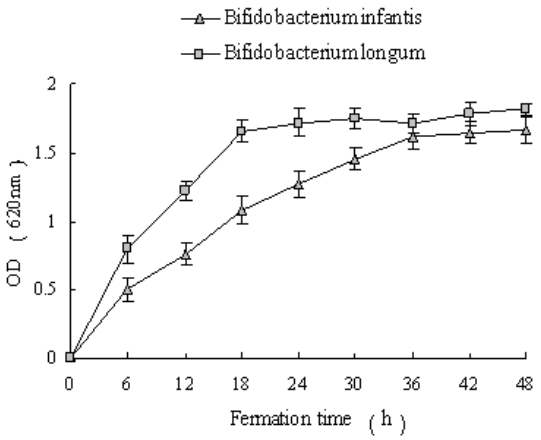

Figure 12. Comparison of growth of *Bifidobacterium infantis* and *Bifidobacterium longum* in enriched medium (SDF 0.2%).

The physiological effects of dietary fiber are highly dependent on the physicochemical properties of the ingested material, such as the WHC, the molecular weight distribution and the viscosity. Meanwhile, the chemical and physical properties of dietary fibers can dictate the degree of fermentability and possibly the composition of intestinal microbiota (Hughes *et al*., 2008). A conclusion was reached by Guillona *et al*. (1998), who described the relevance of the processing of dietary fiber in terms of modifying its physicochemical properties and bacterial metabolism. Decreasing particle size by enzymatic modification increases the surface area of the dietary fiber and hence increases the area exposed to bacteria. Our observations on the physical properties of unmodified and modified sea buckthorn dietary fiber and the *Bifidobacterium* proliferation experiment appear to support this statement.

4. Conclusions

The results of this study indicate that a crude enzyme could be used to increase the SDF content of cereal crops, such as sea buckthorn, and other agricultural plants. Compared with physical methods, the enzymatic process used here increases the yield of SDF and requires less power and fewer resources. In this study, we characterized the physicochemical properties of sea buckthorn fiber and determined the *in vitro* physiological effects on *Bifidobacterium*. Overall, we found that modification by xylanase improved the physicochemical and physiological properties of sea buckthorn dietary fiber.

Enzymatic modification is a mild technique to process dietary fibers and generates the desired products in an efficient manner. A number of studies have reported enzymatic modification of dietary fiber and it is well known that the structure and composition of dietary fiber determine its properties and physiological function. In this study, we determined some of the physicochemical and physiological properties of MDF, but further research of these properties is still necessary. In particular, its potential impact on human health remains to be determined.

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