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A study on synthesis of starch ferulate and its biological properties

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Abstract

Starch esterified with ferulic acid (starch ferulate) was synthesized and its food and biological properties were determined. Starch ferulate showed lower viscosity, higher water-holding capacity, and much less retrogradation during low temperature storage than native starch. It was only partly hydrolyzed (less than 10%) by diastase and the bound ferulic acid was largely released by colonic microorganisms. The rate of release and amount of released ferulic acid were higher than with dietary fibres from wheat bran. Also, it increased survival of two yogurt strains during storage. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Starch; Ferulate physical properties; Fermentation

1. Introduction

Ferulic acid, an effective component of Chinese medicine herbs such as *Angelica sinensis, Cimicifuga heracleifolia* and *Lignsticum chuanxiong* (Fang, 1998; Sakai et al., 1999), is a phenolic acid, ubiquitous in the plant kingdom, which can be absorbed by the small intestine and excreted through the urine (Choudhury, Srai & Dednam, 1999).

Ferulic acid is extensively reported to have preventive effects against several diseases. It can increase the ratio of HDL to VLDL + LDL cholesterol, and bioavailability of vitamin E and decrease total cholesterol in rats (Kamal-Eldin, Frank, Razdan, Tengblad, Basu & Vessby, 2000). It also increases vitality of sperm and is a potential medicine for male infertility (Zheng & Zhang, 1997). Ferulic acid shows strong antioxidant, free radical-scavenging and anti-inflammatory activity, it increases the resistance of LDL to peroxidation, protects LDL cholesterol from oxidation and prevents the oxidative modification of LDL (Castelluccio et al., 1995; Sakai et al., 1999; Zhouen, Side & Wenzhen 1998); it is a good topical protective agent against UV radiation-induced skin damage (Saija et al., 2000).

The most remarkable function of ferulic acid is its anti-tumor and anti-cancer effect. Mori, Kawabata and Yoshimi (1999) reported that the incidence of tongue carcinomas and preneoplastic lesions (severe dysplasia), in rats of the group given ferulic acid in the diet at a dose of 500 ppm after exposure to 4-nitroquinoline- 1-oxide (4NQO) for 5 weeks in drinking water at a dose of 20 ppm, was significantly lower on termination of the experiment (32 weeks) than in the group with the carcinogen alone ($P < 0.005$ and $P < 0.001$, respectively), suggesting that ferulic acid has chemopreventive activity on oral cancer. Several reports have proved that ferulic acid is a potential chemopreventive component for colon cancer (Kawabata et al., 2000; Mori et al., 1999; Taniguchi, Hosoda, Tsuno, Maruta & Nomura,1999; Wargovich et al., 2000).

However, as free ferulic acid does not enter enterohepatic circulation (Chang, Xu, Chen & Feng, 1993), oral or intravenous ferulic acid does not easily reach the colon. Although dietary fibre-bound ferulic acid can get to the colon and is partly released by colon microorganisms, the concentration of released ferulic acid is too low to act as a chemopreventive agent (Ou, Li & Gao, 1999). Enzyme-resistant starch is a type of dietary fibre that is almost completely fermented in the colon (Baghurst, Baghurst & Record, 1996) and may be a satisfactory carrier of ferulic acid. Thus, we use maize starch and ferulic acid to synthesize starch ferulate and test whether this compound can reach the colon and release most of its ferulic acid.

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2. Materials and methods

2.1. Synthesis of starch ferulate

Defatted and deproteinized maize starch were obtained by the Ou, Gao and Li (1999b) method of treating starch with 70% of alcohol, hexane and proteinase.

2.1.1. Synthesis of ferulic acid chloride

5.0 g (AR) of ferulic acid was added to 200ml of $PC1_3$, reacted for 30 min at 65°C by reversed flow, and vacuum-dried at room temperature.

2.1.2. Synthesis of starch ferulate

The ferulic acid chloride was dissolved in 600 ml of dimethyl sulfoxide, added to 100 g of starch and 5 ml of 98% sulfuric acid, continuously stirred, reacted for 40min at 110°C in N_2 flow.

Added to the product mixture was 2400 ml of 95% alcohol, kept at room temperature after strong stirring, centrifuged, washed residue with 70% alcohol and vacuum-dried at 55°C.

2.1.3. Determination of degree of substitution (DS)

Starch ferulate (1.0 g) was extracted with l00 ml mixture solution containing 4% NaOH and 0.5% $NaBH_4$ for 1 h. The extracts were acidified with HC1 to pH 2.5 and extracted three times with 2 volumes of chloroform. The content of free ferulic acid was determined spectrophotometricatly at 320 nm by a UV-260 model violet-visible spectrophotometer (Rybka, Sitarski & Racznska-Bojaowska, 1993), with chloroform as blank. DS was calculated by difference.

2.2. Determination of physical properties of starch ferulate

Viscosity of starch ferulate: Defatted and deproteinized maize (2.5%) starch and 2.5% of aqueous starch ferulate solution were used to test viscosity by using a NDJ-1A rotational model viscometer at 25°C.

2.2.1. Retrogradation test

Defatted and deproteinized maize starch (2.5 g), starch ferulate (2.5g) and the mixture of both (2.5 g of each) were respectively dissolved in 100 ml of deionized water, kept at 4°C for 48 h, centrifuged (4000 ×) for 20 min, residue dried in a 105°C autoclave, and retrograded starch was obtained.

2.2.2. Water-holding capacity

A sample (2.0 g) was added to 50 ml deionized water, slowly stirred for 20 min at room temperature, centrifuged, and the residue weighed. Water-holding capacity = residue (g)/2.0.

2.3. Biological properties of starch ferulate

2.3.1. Hydrolysis by diastase

Defatted and deproteinized maize starch (100 ml of 2.5%) and 2.5% starch ferulate were prepared in pH 6.00, 0.05mol/l phosphate buffer, 0.1 g diastase (6000U) was added, and kept in water bath at 37°C. The glucose content in 2.0 ml was determined after 30, 60, 90, 120 and 180 min according to the hexokinase method of Yokoyama et al. (1997).

The activity of diastase was determined by the dinitrosaticylic acid (DNSA) method according to Benhura et al. (1999). 1 U = 1 μmol maltose produced by 1 mg diastase in 1 min at 25°C.

2.3.2. Fermentation of starch ferulate by human colon microorganiosms

Starch ferulate (1.0 g), along with 1.0 g of water-insoluble and water-soluble dietary fibre from wheat bran as controls were, respectively, added to 100 ml of human colon microorganism fermentation solution and fermented at 37°C according to Ou, Gao & Li (1999). 3-ml samples, at different time intervals, were collected, centrifuged (4000× for 20 mm) three times (by adding deionized water after first centrifugation), mixing the supematants, acidified with HC1 to pH 2.5 and extracted three times with 2 volumes of chloroform; the released ferulic acid was determined according to Rybka et al. (1993).

2.3.3. Effect of starch ferulate on the growth and viability of yogurt bacteria

Components of medium were: milk powder 8%, defatted milk powder 2%, sucrose 6%, starch ferulate 1% or 1% maize starch as a control. Three percent of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* mixtures were inoculated and kept at 43°C to produce yogurt. The yogurts were kept at 4°C and viable bacteria were tested at 2, 4, and 6 weeks. The count of viable bacteria was determined by plate culture.

2.4. Nitrite scavenging activity of colon microorganisms fermentation solution of starch ferulate

Fermentation solution (10 ml) of starch ferulate by human colon microorganisms at 12 h was centrifuged; 5 ml supernatant mixed with 95 ml of 1000 mol/l sodium nitrite at pH 7.0; 2 ml samples were collected at 5, 10, 20, 30 and 60 min and free NO_2^- was tested according to Moller, Dahl and Bockman (1988). Scavenging amount of nitrite = [1000 (mol/l)× 95 (ml)− concentration of free NO_2^- (mol/l)× remaining volume of reactive solution after sampling (ml)]÷ volume of supernatant added (5 ml) ÷ 1000.

Solutions of starch ferulate and the prepared maize starch mentioned above were prepared as follows:

starch was gelatinized and starch ferulate was dissolved with 50–60 of deionized water, and cooled. All the tests were replicated three times.

3. Results

3.1. Physical properties of starch ferulate

Starch ferulate obtained in our experiment contained 4.2% ferulic acid with DS 0.036. When maize starch was esterified by ferulic acid, the physical properties changed to some extent. Its viscosity decreased slightly but the water-holding capacity increased significantly (Table 1), suggesting that it is beneficial for increasing water-holding capacity of intestinal digesta and protecting against constipation. Another significant change of starch by esterification of ferulic acid is its significant decrease in retrogradation; furthermore, starch ferulate reduced retrograded starch when added to native starch (Table 1).

3.2. Effect of diastase on hydrolysis of starch ferulate

Diastase is a complex enzyme composed of α-mylase, β-amylase and glucoamylase; hydrolysis of starch by diastase is regarded as similar to the situation in human small intestine than any one single amylase. The results show that much less starch was hydrolyzed by diastase compared with its native form (Table 2). Calculating from data in Table 2, less than 10% starch was hydrolyzed by diastase, suggesting that most intaken starch ferulate could escape hydrolysis in the small intestine. Thus, starch ferulate ingested would reach the colon as dietary fibre. Furthermore, it has two advantages compared to dietary fibre. The first is that its water-holding capacity is higher, and second, it contains much more ferulic acid than dietary fibre, wheat bran fibre, for example, only contains 0.4% (Ou, Li & Gao, 1999).

3.3. Release of ferulic acid from starch ferulate by colon microorganisms

An in vitro experiment was used to investigate whether ferulic acid can be released from starch ferutate by human colon microflora. The results show that the release rate and amount of ferulic acid were much higher than with dietary fibres from wheat bran (Table 3).

According to Neut et al. (1997), nitrite was produced in the colon by colon bacteria that would cause inflammation, such as colitis. Our results show that the

Table 1
Some physical properties of starch ferulate[a]

Properties (%)	Viscosity (Pa.S)	Water-holding capacity (g/g)	Retrograded starch (g/g)
2.5 Maize starch	6.4±0.1b	6.4±0.1a	0.67±0.02c
2.5 Starch ferulate	5.3±0a	15.6±0.2c	0.05a
2.5 Maize starch + 2.5 Starch ferulate	8.9±0.1c	10.7±0.1b	0.34±0.01b

[a] Values (means±S.D. $n=3$) with different letters within a colunm are significantly different at 5% level.

Table 2
Hydrolysis of starch ferulate by diastase[a]

	Glucose released (mg glucose)				
	30 min	60 min	90 min	120 min	180 min
Maize starch	156±0.5a	289±1.1a	433±0.9a	589±1.5a	713±1.9a
Starch ferulate	50.2±1.0b	98.7±0.8b	101±0.7b	99.4±1.3b	100±1.0b

[a] Values (means±S.D., $n=3$) with a different letter within a column are significantly different at 5% level.

Table 3
Release of ferulic acid from starch ferulate by colon microflora in vitro at different times[a]

Treatment	Ferulic acid release (%)					
	4 h	8 h	12 h	16 h	20 h	24 h
Starch ferulate	22.8±0.2a	47.4±.4a	78.3±0.5a	94.5±0.7a	96.8±0.4a	98.2±0.5a
WIDF[b]	7.8±0.3c	21.2±0.4c	34.5±0.6c	43.8±0.4c	53.6±0.5c	63.9±0.3c
WSDF	9.6±0.2b	24.3±0.3b	43.5±0.6b	66.4±0.5b	79.5±0.3b	84.2±0.4b

[a] Values (means±S.D., $n=3$) with a different letter within a column are significantly different at 5% level.
[b] WIDF, water-insoluble dietary fibre from wheat bran; WSDF, water-soluble dietary fibre from wheat bran. WIDF and WSDF were prepared according to Ou et al. (1999)

Table 4
Effect of starch ferulate on reproduction and survival of yogurt bacteria

Treatment		Count of bacteria (cfu/g)			
		0 week	2 week	4 week	6 week
Maize starch	*S. thermophilus*	2.3×10^8	1.9×10^8	1.2×10^8	8×10^7
	L. bulgaricus	3.8×10^7	3.3×10^7	2.6×10^7	1.3×10^7
Starch ferulate	*S. thermophilus*	2.2×10^8	2.3×10^8	2.1×10^8	1.9×10^8
	L. bulgaricus	3.7×10^7	3.7×10^7	3.5×10^7	3.4×10^7

fermentation solution of starch ferulate showed high nitrite-scavenging activity. A maximum scavenging amount, 24.9 μmol/ml was reached in 10 min, suggesting that starch ferulate may be a potential chemopreventive compound against colon inflammation.

3.4. Effect of starch ferulate on reproduction and viability of yogurt bacteria

Yogurt is a health food. Yogurt bacteria were used to test the effect of starch ferulate on their reproduction and viability. Results showed that reproduction of *S. thermophilus* and *L. bulgaricus* was slightly decreased but survival viability was significantly increased by addition of starch ferulate compared with maize starch (Table 4).

4. Discussion

Colon health care seems to have become very important in modern society as more and more products for colon health, such as active bacilli, active bifidobacteria and prebiotic factors (oligosaccharides, enzyme-resistant starch) are popular in the consuming market. However, Amann, Kullen and Martini (1998) reported that bifidobacteria would not increase by exogenous bifidobacteria intake, suggesting that the main pathway to increase probiotic bacteria in the colon is not by exogenous probiotic intake, but by prebiotic intake, such as dietary fibres and oligosaccharides.

For increase in colon probiotic bacteria, another pathway is to deliver health components safely to the colon. This is our objective with a preliminary attempt to synthesize starch ferulate. Extensive toxicological and biological experiments are needed to test this kind of product.

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