Stability of prebiotic, laminaran oligosaccharide under food processing conditions

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Abstract. Prebiotic stability tests on laminaran oligosaccharide under food processing conditions were urgently performed to determine the ability of prebiotics deal with processing. Laminaran, oligosaccharide is produced from enzymatic hydrolysis. To further apply this prebiotic, it is necessary to test its performance on food processing. Single prebiotic or in combination with probiotic can improve human digestive health. The effectiveness evaluation of prebiotic should be taken into account in regards its chemical and functional stabilities. This study aims to investigate the stability of laminaran, oligosaccharide under food processing condition.

1. Introduction
Several species of pathogenic bacteria have been found to cause acute gastroenteritis, ulcerative colitis, colon cancer, and pseudomembranous colitis [1], while certain beneficial genera such as bifidobacteria and lactobacilli are known to provide protection against infection [2]. Prebiotic is non digested carbohydrate which promote the growth of good bacteria in the intestine [3].

Non-digested carbohydrate and from the algae has an important health supporting effect. They are bowel function, preventing colon cancer, promoting the growth of certain beneficial microorganisms, lowering serum cholesterol, increasing mineral absorption, edema reducing agent, and antihyperglycemic [4,5]. Generally, commercial prebiotics are produced by terrestrial plant oligosaccharides. Commercial prebiotics often cause consumer anxiety due to the excessive amount of released gas during rapid fermentation in the digestive tract [6]. The majority of prebiotics producing gas are commercial prebiotics fermented by microorganisms [7].

Brown algae are sources of several bioactive polysaccharides such as alginate, FCP, and laminar [4, 8, 9, 10]. Seaweed or algae, according to Chamidah et al. [11], show the effects of prebiotics as antibacterial. Laminaran is a component of algae polysaccharide that may have prebiotic activities. stating that laminaran polysaccharides could not be digested by digestive enzymes. About 80% of laminar are fermented by human fecal bacteria after 24 hours [12,13,14, 15].

Prebiotics have been added to yogurt and other fermented dairy products, as well as a variety of other foods such as biscuits, candies, cereals, dairy products, beverages, baby food formulas and weaning foods [16,17]. However, data on prebiotic stability during food processing in complex matrices are very limited [18, 19].

Food processing affects prebiotics, either in quantity or conformation. Food processing and cooking operations change the concentration of prebiotics in food. Prebiotic stability during food processing is an important requirement as their biological activity may depend on their structural
integrity. For example, hydrolyzed or degraded prebiotics caused by food processing may no longer be active [16, 20]. Therefore, it is important to know the effects of treatment and treatment on prebiotic carbohydrates to maintain optimum prebiotic concentration in processed foods as an effort to maintain intestinal health. The objective of this study was to investigate the stability of the laminaran under food processing condition.

2. Methodology

2.1. Material

Prebiotics used in this study were crude laminaran by adopting a method described by Huebner et al. [21] and Moore [22] with slight modifications. The chemicals used include 96% ethanol, H₂SO₄, HCl (technical) and aquadest. Reagents used for chemical analysis were anhydrous glucose, Nelson reagent, aluminum hydroxide slurry, and glucose test/ Glucose Oxidation Method, acetone and chloroform, ethanol, CaCl₂, HCl, Na₂CO₃, and polyvinylpyrrolidone (C₆H₉NO)n (Merck, with grade pro analysis, pa). Whatman no 40 paper, laminaran standard from Laminariadigitata and laminariaceae enzyme from Trichodermasp (Sigma-Aldrich), endo enzyme (1,3)- β-D glucanase from Trichoderma viridae (Megazyme) were also used.

2.2. Preparation of laminaran oligosaccharide

Crude Laminar was enzymatically hydrolyzed using endo (1,3) -β -D-glucanase enzyme from Trichoderma viride. This hydrolysis procedure referred to Bohm et al. [23] and Critenden and Playne [24]. Hydrolysis was carried out at 45°C. This study was conducted using a simple randomized design with one level of hydrolysis 0, 5,10, 15, 20, 25, and 30 mins. As shown in Figure 1, after obtaining the optimum time for the laminar oligosaccharide manufacture, a process for obtaining short chain laminar oligosaccharides by combining Sudarmo et al. [25] and Alderkamp et al. [26] with slight modifications was conducted (figure 1).

![Figure 1. Process of crude laminaran hydrolysis.](image-url)
Enzymatic Hydrolysis, 37°C

Hydrolysis time 20 minute

Plus 100°C ethanol so that the final concentration of 80%

3500 rpm centrifugation

Plus 96% ethanol (1:5)

In idle for 3 days

3500 rpm centrifugation

Figure 2. Production Oligosaccharides Laminaran.

2.3. Data Analysis
The obtained quantitative data were tested by using analysis of variance (ANOVA) and then continued by Duncan's test ($\alpha=0.05$) using SPSS 16.0 for Windows application program and Statistic Version 6 program.

2.4. Stability test procedure of laminaran oligosaccharide in food processing conditions
Stability test procedure on processing conditions was displayed on figure 3-4.
3. Results and Discussion
After hydrolysis for 20 minutes with Endo-(1,3)-β-D-Glucanase enzyme from Trichoderma sp. and monosaccharide was discharged, both OLAE and OLME were obtained (data not shown).
3.1. Low pH effect

![Graph showing reduced sugar levels of laminaran oligosaccharide at low pH effects.]

Figure 5. Reduced sugar levels of laminaran oligosaccharide at low pH effects.

Food material must be stable during food processing. Low pH stability is important for the prebiotic since they will experience low pH in the digestive tract. Prebiotics may undergo chemical changes, but their functional activity may remain or even increase after processing [16]. The results for the stability test were shown in figure 6.

Chemical degradation was not detected in the test. Laminaran showed its stability even at the pH of 3. Reduced sugar content between OLAE and OLME is very significant (p <0.05), OLAE <OLME. This is because OLME contains larger carbohydrate components (laminaran) that are more easily degraded by acid, thus producing higher reducing sugars. The results of this study indicated a similar trend with the result of [16,27,28] which used four commercial prebiotic samples. Thus, both OLAE and OLME are stable at low pH treatments.

3.2. Heat effect and low pH

During food processing, food material will contact with the heat. Hence, such condition needs to be tested on prebiotic candidates. The test is feasible to be applied to determine the biological stability in food material when exposed to heat. The result of heat and low pH treatment could be seen in Figure 6.
Figure 6. Reduced sugar content of laminaran oligosaccharide at 85 °C and low pH.

The laminaran still stable under heat and low pH. When compared with that of commercial inulin prebiotics, the reduction value of OLME laminaran oligosaccharide is higher. The decrease of sugar in this inulin prebiotic is due to biological matrial degradation when inulin is exposed to acidic and moderate heat (70 °C) [29]. Crittenden and Playne [30] showed that heated inulin at 195 °C for 30 mins resulted in perfect degradation of the fructant chain and new products with lower molecular weight were obtained. Reduced sugar level of OLAE and OLME is relatively unchanged during the food processing treatment. This means that the laminaran oligosaccharides are relatively stable against heat and low pH.

3.3. Browning calculation
Calculation of its relative browning percentage in several laminaran types was shown in table 1.

Table 1. Relative percentage of browning laminaran and inulin oligosaccharides.

| Substrat      | PersenRelatif Browning |
|---------------|------------------------|
|               | 0 hour | 1 hour | 2 hour | 3 hour |
| OLME /Glc 10% | 340    | 369    | 373    | 380    |
| OLAE /Glc 10% | 24     | 26     | 26     | 29     |
| INULIN/Glc 2% | 44     | 42     | 36     | 37     |

It could be inferred from table 1 that the degradation during browning process is very high for OLME substrates compared to OLAE ones, moreover when compared to inulin, which is a tested as a commercial prebiotic.

4. Conclusion
Laminaran in the form of both OLAE and OLME are stable against the effect of low pH treatment, where the performance of OLME is better than OLAE. Laminaran in the form of OLAE and OLME is stable to heat and low pH but not resistant to Maillard reactions.

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