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Kinetic studies for the preparation of probiotic cabbage juice: Impact on phytochemicals and bioactivity

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ABSTRACT

A kinetic study for the production of probiotic cabbage juice was carried out under controlled pH and dissolved oxygen using several strains of lactic acid bacteria (LAB). Furthermore, effect of probiotic fermentation on polyphenolic content and antioxidant capacity was investigated. Results showed significant growth in *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* corresponding to 9.19, 9.47 and 10.6 log CFU/ml after 24 h of fermentation, which is satisfying criteria for a food product to be characterized as probiotic. Lactic acid (LA) was the major end product of the fermented cabbage juice attaining the concentrations of 6.97, 9.69 and 12.2 g/l LA for *L. plantarum*, *L. rhamnosus* and *L. brevis*, respectively. LAB fermentation retains more than 75% of total phenolic content (TPC) and total flavonoid content (TFC) of the initial raw material, and similar set of results were observed for antioxidant capacity. First-order kinetics model fitted well with the experimental data with R^2 value ranging from 0.92 to 0.96, 0.96 to 0.98 and 82.2 to 97.2 for TPC, TFC and antioxidant capacity, respectively. During refrigerated storage (4 °C), all the probiotic cultures met the criterion of maintaining counts greater than 8 log CFU/ml; in addition to maintaining bioactive components and antioxidant capacity.

1. Introduction

Foods which promote health beyond providing basic nutrition are termed as 'functional foods'. These foods have the potential to promote health in ways not anticipated by traditional nutritional science (Beganović et al., 2011). Modern consumers are showing continuously a remarkable interest in functional foods with more emphasis in recent times on probiotic types of products. The main motive for purchasing functional foods is the growing desire to use foods either to help prevent chronic illnesses or to optimize health. During the past two decades there has been a large increase in the worldwide sales of functional products containing probiotic bacteria which led to one of the fastest growing food sectors, with a compound annual growth rate of 8.6% in the 10 years to 2012 (Khan et al., 2013).

Available literature confirms that the addition of probiotics to food provides several health benefits, including reduction in the level of serum cholesterol, improved gastrointestinal function, enhanced immune system, antimutagenic property, anti-carcinogenic property, anti-diarrheal property, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection by the addition of selected strains to food products (Agerholm-Larsen et al., 2000; Gotcheva et al., 2002; Nomoto, 2005; Sindhu and Khetarpaul, 2003).

Probiotics have successfully been added to a wide range of dairy based food products. However, the problem of lactose intolerance and cholesterol content has increased the demand for non-dairy based probiotic products. About 5–15% of the Europe population is lactose intolerant and this number increased up to 80% in some part of the world such as central Asia and Africa (de Vrese et al., 2001). Dairy products with probiotic bacteria are unsuitable for this group of population because of its health condition. Additionally, with growing awareness of gut health, consumers demand a wider variety of probiotic products beyond dairy based food products. This work is part of an on-going project to evaluate the potential of Brassica vegetables for the development of a probiotic-based product. Fermentation is widely used in the food industry to improve the sensory characteristics of a product as well as to eliminate certain undesirable constituents, make nutrients more accessible while preserving and even improving the nutritional properties. In a previous study, it was reported that cabbage juice is a good medium for the growth of probiotics (Yoon et al., 2006). It was also observed that natural fermentation of cabbage in the production of sauerkraut increased the initial antioxidant (AO) activity which could have resulted from the combined effects of wounding and chemical processes incurred by lactic acid bacteria (LAB) (Kusznierewicz et al., 2008).

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Furthermore, there is now an increasing interest in modelling the kinetics of beneficial microorganisms in food systems leading to a better understanding of the fermentation process. Mathematical models can help to predict the influence of fermentation operating parameters on the rate of substrate utilization, cell growth and lactic acid (LA) production (Biazar et al., 2003). The use of these models may lead to the development of better strategies for the optimization of the fermentation process to ensure its economical viability. Therefore, in the present report, a kinetic study for the production of probiotic cabbage juice was carried out using several strains of LAB in order to achieve maximum LAB in cabbage juice. At the same time, the effect of fermentation on inherent total phenolic content (TPC), total flavonoid content (TFC), and AO capacity of cabbage juice was also studied. Furthermore, shelf life of fermented probiotic cabbage juice was undertaken by evaluating the cell viability, lactic acid content, pH and phytochemical constituents.

2. Materials and methods

2.1. LAB strains and inoculum preparation

Lactobacillus plantarum ATCC 8014; *Lactobacillus rhamnosus* ATCC 9595 and *Lactobacillus brevis* ATCC 8287 were purchased from Medical Supply Company, Dublin, Ireland. The culture was maintained at −70 °C in 20% glycerol stocks and grown in de Man, Rogosa and Sharp (MRS) broth (Scharlau Chemie, Barcelona, Spain) at 37 °C. For the preparation of inoculum, 25 ml of sterile MRS broth was inoculated with 1 ml of thawed stock culture and incubated at 37 °C for 12–14 h. This was then serially diluted 100 times to obtain working culture containing 5–6 log colony-forming unit (CFU)/ml cells as determined by plate counts.

2.2. Plant materials and preparation of juice

Fresh white cabbage (*Brassica oleracea var. capitata*) was purchased from a local supermarket in Dublin. Twenty five to thirty white cabbage heads (45–50 kg) were randomly selected and trimmed of their outer leaves and the stem. The heads were then divided into four segments, and the central core was removed. The segments were chopped into small pieces using an ordinary knife. The part of shredded cabbage was blended with the addition of water (1:1 w/v), and the juices were squeezed out from the pulps and sterilized for 15 min at 121 °C in an autoclave (Tomy SS-325, Tomy Seiko Co. Ltd, Tokyo, Japan) and stored under dark refrigerated conditions (4 °C). The sterilized juice was filtered through sterilized muslin cloth and further diluted with sterile double distilled water (2:1 v/v) (hereafter the diluted cabbage juice was called WCJ) and 5 l of WCJ was used for each batch of fermentation.

2.3. Preliminary LAB fermentation

In order to check the applicability of WCJ as a substrate for LAB growth and to compare the growth pattern in WCJ with typical medium for LAB cultures, preliminary experiments involved screening of WCJ (diluted with water, 2:1) and MRS broth. The preliminary LAB fermentation was performed in a 96-well round-bottom microplate (Sarstedt, Inc, USA). The sterilized WCJ was inoculated with various LAB strains (5% v/v), and MRS was inoculated in similar fashion and 200 μl dispensed in each microtiter well. Wells containing sterile MRS or WCJ (200 μl) were treated as blanks to check for contamination. The LAB growth was monitored at 600 nm using the micro plate spectrophotometer (Powerwave, Biotek, VT, USA) (preceded with 30 s agitation) over 24 h at 30 min intervals. Growth curves of the test organisms were analyzed graphically as a plot of OD_{600} versus time. Maximum OD_{600} (OD_{max}) obtained and lag time (λ) for each growth curve were calculated using Gen5 reader data analysis software.

2.4. Fermentation in 7 l bioreactor under controlled pH

Seed culture (200 ml) was prepared as mentioned in Section 2.1 (LAB strains and inoculum preparation). Cultivation was carried out at 37 °C at the agitation speed of 200 rpm, in a 7 l Bioflo 415 bioreactor (New Brunswick Scientific Ltd.) containing 5 l of WCJ under aseptic conditions. The bioreactor was sterilized *in situ*, cooled and then inoculated with 5% inoculum (v/v). Culture pH was maintained at 7.0 by the automated control system of bioreactor with the addition of acid or base. Samples were withdrawn at 3–4 h interval and analyzed for viable cell count, lactic acid (LA) production, phytochemical constituents and AO capacity.

2.5. Viable cell counts

Viable cell counts in the fermented WCJ (log CFU/ml) were determined by the standard plate method with MRS medium. Dilution of 1 ml broth was carried out in 9 ml maximal recovery diluent (MRD) to plate the suitable dilution. The plates were incubated at 37 °C for 36–48 h, for cell enumeration.

2.6. Effect of cold storage on probiotic WCJ

After 24 h of fermentation at 37 °C, the fermented WCJ was stored at 4 °C for four weeks. Samples were taken at three-day intervals, and pH, lactic acid, viable cell count, phytochemical content and AO capacity were estimated.

2.7. Analytical procedure

Each sample of the fermented broth was centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was used for the analysis.

2.7.1. Total phenolic and flavonoid contents

TPC and TFC of samples were estimated according to our earlier report (Jaiswal et al., 2012b). In brief, for the TPC estimation, 100 ml aliquot of sample in deionized water were mixed with 2 ml of 2% Na_2CO_3 and were allowed to stand for 2 min at room temperature. After incubation, 100 ml of 1 N Folin-Ciocalteau's phenol reagent was added. Reaction mixture was allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Genesys 20; Thermo Spectronic, Madison, WI). Results were expressed as μg gallic acid equivalents (GAE) per ml of sample.

For the TFC estimation, 250 ml of sample was mixed with 1.25 ml of deionized water and 75 ml of 5% $NaNO_2$ solution. After 6 min, 150 ml of 10% $AlCl_3 \cdot H_2O$ solution was added. Finally, 0.5 ml of NaOH (1 M) solution was added and the total volume was made up to 2.5 ml with deionized water. Absorbance against blank was taken at 510 nm using a spectrophotometer. Results were expressed as μg quercetin equivalents (QE) per ml of sample.

2.7.2. Determination of sugar, organic acids and protein contents

Determination of individual sugar content and organic acids was carried out as described in our earlier report (Jaiswal et al., 2012a). Standards for the organic acids such as lactic, propionic, citric, oxalic and acetic acid; sugars such as glucose, fructose and arabinose were used to identify and quantify the contents in the samples. Protein concentration of probiotic cabbage juice was estimated using Bradford's method (Bradford, 1976). In brief, 200 μl aliquot of sample was mixed with 800 μl of Bradford's reagent (Sigma–Aldrich, Germany) and allowed to stand for 5 min at room