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Kinetic Studies for the Preparation of Probiotic Cabbage Juice: Impact on Phytochemicals and Bioactivity

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Highlights

- Kinetic studies for the preparation of probiotic cabbage juice were carried out.
- Significant growth in LAB was observed in white cabbage juice.
- The Gompertz model was applied to predict probiotic growth in white cabbage juice.
- Lactic acid was the major end product of the fermented cabbage juice.
- LAB fermented cabbage juice retains more than 75% of phytochemicals and bioactivity.

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 0.92-0.96, 0.96-0.98 and 82.2-97.2 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.

Keywords: Antioxidant capacity; Kinetic study; Lactic acid; Polyphenols; Probiotics; cabbage juice

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 to 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).

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

L. 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 five litre 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 sec 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 litre Bioflo 415 bioreactor (New Brunswick Scientific Ltd.) containing 5 litre 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 1N 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 (1M) 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 temperature. Absorbance of all the sample solutions was measured at 595 nm using a spectrophotometer (Genesys 20; Thermo Spectronic, Madison, WI). Results were expressed as µg bovine serum albumin equivalents (BSAE) per ml of sample.

2.7.3 Antioxidant capacity

Two different methods [2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) and Ferric reducing AO potential (FRAP) assay were used for the estimation of AO capacity of the sample (Rajauria et al., 2012). DPPH scavenging capacity assay was performed in a 96-well round-bottom microplate with 1:1 ratio of 100 µl of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) radical solution (165 µM) and 100 µl of test sample. The DPPH solution was freshly prepared in methanol . The reaction mixtures were incubated for 30 min at 25°C in dark conditions, and absorbance measured at 517 nm in a microplate reader (Biotek

EL808 Microplate Reader, BioTek Instruments, Inc., VT 05404, USA). Results were expressed as μg ascorbic acid equivalents (AscE) per ml of sample.

For FRAP analysis, reagent was freshly prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) (Sigma-Aldrich, Germany) in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride (Sigma-Aldrich, Germany). All solutions were used on the day of preparation. The reaction was performed in a microplate reader with 96-well plates (Sarstedt, Inc, USA) at a temperature of 37°C. Preheated 100 μl FRAP reagent at 37°C was dispensed in each well with 50 μl of sample or standard. The reagent blank assay was performed by using 100 μl FRAP reagent and 50 μl of water. The absorbance was read after 10 min at 593 nm with the help of microplate spectrophotometer. Trolox (Sigma-Aldrich, Germany) was used as a standard and the results were expressed as μg trolox equivalents (TE) per ml of sample.

2.8 Mathematical modelling

Modified Gompertz equation (Zwietering et al., 1990) was fitted to the logarithm of the cell concentration in order to estimate the maximum specific growth rates of *L. plantarum*, *L. rhamnosus* and *L. brevis*. The model is described by the equation (Eq. 1):

$$\log\left(\frac{N}{N_0}\right) = A \times \exp\left\{-\exp\left[\frac{\mu \times e}{A}(\lambda - t) + 1\right]\right\} \qquad \text{(Eq. 1)}$$

where μ_{max} is the maximum specific growth rate of cell population, A is the log increase in population and λ is a delayed time variable (lag phase), N is the CFU/ml at any time t, N_0 is the initial CFU/ml.

The fermentative production of LA was described by the following equation (Eq. 2) (Mercier et al., 1992).

$$\frac{dP}{dt} = P_r \times P\left(1 - \frac{P}{P_m}\right) \qquad \text{(Eq. 2)}$$

Where t is time, P is LA concentration (g/l), Pm is the maximum concentration of lactic acid (g/l), Pr (h^{-1}) is the ratio between the initial volumetric rate of product formation (rp) and the initial product concentration P_0. Eq. (2) can be solved to give the following expression:

$$P = \frac{P_0 P_m e^{P_r t}}{P_m - P_0 + P_0 e^{P_r t}} \qquad \text{(Eq. 3)}$$

The value of model parameters for (A, μ, λ. P_0, Pm and Pr) were fitted by with a Marquardt algorithm, using the STATGRAPHICS Centurion XV (StatPoint Technologies, Inc., Warrenton, VA) statistical software.

The kinetics of WCJ phenolic content, flavonoid content and different AO capacity were described by fitting a zero order (Eq. 4), first-order (Eq. 5) or a the second order kinetic model (Eq. 6) to the experimental data

$$A = A_0 \pm kt \qquad \text{(Eq. 4)}$$

$$A = A_0 \times \exp(\pm kt) \qquad \text{(Eq. 5)}$$

$$\frac{A}{A} = \frac{A}{A_0} \pm kt \qquad \text{(Eq. 6)}$$

Where, A is the parameter to be estimated, the sub index 0 indicates the initial value of the parameter, t is the fermentation time, and *k* is the rate constant at temperature T. For the

parameter estimation, the individual measured concentrations were used instead of mean values of duplicate or triplicate experiments, thus taking into account variability within the samples.

2.9 Statistical analysis

Fermentation was carried out in duplicate whereas all the other analysis were carried out in triplicate and replicated twice unless otherwise stated. Results were expressed as mean values ± standard deviation. The regression analysis and analysis of variance (ANOVA) were carried out using the STATGRAPHICS Centurion XV software. Values of $P < 0.05$ were considered as statistically significant. The coefficient of determination (R^2) and mean square error (MSE) were used as criteria for adequacy of fit.

3 Results and discussion

3.1 Initial fermentation studies with microtiter plate reader

Prior to the application of bioreactor fermentation using 5 litre of WCJ, preliminary fermentation was carried out to check the applicability of WCJ as a substrate for LAB growth. LAB strains have complex nutritive requirements, and therefore, they are usually associated with nutrient-rich environments such as animal bodies, plants, and foodstuffs. LAB grew well in the WCJ without additional supplements, suggesting that WCJ comprises a good medium for the growth of LAB strains.

The OD_{max} obtained by the studied LAB strain in WCJ were in the range of 1.05 to 1.67, these values are higher than those reported in other studies for cabbage and other vegetable juice (Champagne et al., 2009; Savard et al., 2003). The higher growth of LAB observed in this study could be related to the nutritional content of Irish white cabbage. Among the LAB strains *L. rhamnosus* attained highest growth level (OD_{max} 1.67) followed by *L. plantarum* (OD_{max} 1.48)

and *L. brevis* (OD_{max} 1.05). Though, OD_{max} values were always lower in WCJ compared to MRS.

Generally, MRS medium has phosphate buffers, which are not available to WCJ, however, it is anticipated that higher OD_{max} readings observed in MRS are due to a higher buffering capacity of MRS, rather than a nutrient deficiency of WCJ. Nonetheless, the results obtained in this study were encouraging as LAB grew satisfactory in the WCJ. Published literature shows that controlling the pH is critical in obtaining high biomass yields (Cerning et al., 1994). Therefore, in order to produce large volume (5 litre) of WCJ with high biomass, a further study was carried out in the bioreactor under controlled pH and dissolved oxygen.

3.2 Fermentation under controlled conditions using 7 litre bioreactor

3.2.1 Growth of LAB during fermentation

The time course for the growth of *L. plantarum, L. rhamnosus* and *L. brevis* is presented in Fig. 1. Total fermentation time ranged from 32-36 h and was dependent on the time of entering the decline phase for the LAB strains under study. However, all of the LAB strains showed different growth characteristics but reached the stationary phase within 24 h after which there was no significant increment observed in LAB growth. Initially, the concentration of probiotic bacteria was approximately 5 log CFU/ml; after 24 h of fermentation it increased to 9.19, 9.47 and 10.6 log CFU/ml for *L. brevis*, *L. plantarum* and *L. rhamnosus*, respectively. These results were in agreement with OD_{max} data in the preliminary studies. It is anticipated that WCJ sustains the growth of probiotic bacteria due to protein content, total available sugars and micronutrients. In a previous report, it was found that cabbage is among the nutritionally well-balanced vegetables; it is rich in proteins, vitamins, carbohydrates, sugars (including fructose, glucose, sucrose and

raffinose), essential n-3 fatty acid and linolenic acid (Batista et al., 2011). Thus, it is presumed that the bacteria utilized these nutrients as an energy source without additional nutrient supplementation. Growth of *Lactobacillus* in vegetable juices, including cabbage juice have been studied by various authors (Champagne et al., 2009; Gardner et al., 2001; Tolonen et al., 2004; Yoon et al., 2006) and maximum cell concentration has been reported to vary from log 7 to 8 CFU/ml.

In a previous study, it was found that *L. plantarum, L. casei* and *L. delbrueckii* grew rapidly in sterilized cabbage juice without nutrient supplementation reaching nearly 8 log CFU/ml after 48 h of fermentation at 30°C (Yoon et al., 2006). Similarly, some other authors investigated the possibility of producing a functional carrot juice using *L. rhamnosus* and *L. bulgaricus* and a growth of 5×10^9 CFU/ml after 48 h was reported (Nazzaro et al., 2008). However, the results of the present study were encouraging as the time required to reach more than 9 log CFU/ml viable cell count was shorter (24 h).

There are several models, which have been used to describe the sigmoid growth curve of a microorganism such as the Baranyi, Gompertz or Logistic models (Zwietering et al., 1990). Gompertz model is regarded as the most suitable to describe such microbial growth curves due to its simplicity and the low correlation, or interdependence, of the characteristic parameters. In this study, the lag phase duration as obtained from the Gompertz equation was minimal (less than 3 h) and was the magnitude of 1.27, 1.99 and 2.81 for *L. plantarum L. brevis*, and *L. rhamnosus*, respectively (Table 1). Among the probiotic cultures, *L. rhamnosus and L. brevis* grow at a significantly faster rate than *L. plantarum* (Table 1). The specific growth rate (μ_{max}) of *L. rhamnosus* and *L. brevis* was 1.5 times higher as compared to *L. plantarum*. The high growth

rate resulted in a faster consumption of nutrients and thus the stationary phase for *L. rhamnosus* was attained in a total fermentation time of 16 h. *L. brevis* and *L. rhamnosus* showed 1.4 times higher specific growth rate (μ_{max}) value as compared to *L. plantarum.*

Glucose was the main sugar present, accounting for ≈55% of the total sugar while fructose accounted for almost 45%. This result is in line with previous findings, demonstrating that glucose and fructose are the main sugars present in white cabbage (Rosa et al., 2001). It is worth remarking that the sugar content reduced continuously as the bacterial population increased throughout the fermentation time. It was observed that fermentation led to a sharp depletion in the glucose and fructose levels in WCJ. Both the glucose and fructose were simultaneously consumed by the LAB strains during the fermentation process, but the rate of depletion was higher for glucose and more intense, which is usually because of the glucophilic nature of LAB. In the case of *L. plantarum*, after 24 h of fermentation only 33% residual glucose was left, while in the case of *L. brevis* it was 37% and for *L. rhamnosus* there was 10% remaining. A similar trend was also shown by fructose; however, the consumption was lower than glucose as after 24 h of fermentation only 76, 50 and 43% of fructose was consumed by *L. plantarum L. brevis*, and *L. rhamnosus*, respectively. As a result, the residual fructose concentrations were considerably higher than the residual glucose concentrations after fermentation.

3.2.2 pH and organic acid production during fermentation

Initially, the pH of the WCJ was 6.2, which was adjusted to 7.0 before inoculation. As the fermentation was carried out under controlled pH conditions, further pH was adjusted by adding 1M HCL or 4M KOH. The pH decreased slowly for the first 4 to 6 h of fermentation, and then dropped more quickly until LAB was in the decline phase. The drop in pH is due to the

production of organic acids. Theoretically, the fermentation of 2 mol of glucose by LAB produces 3 mol of acetate and 2 mol of lactate via the fructose-6-phosphate shunt. The proportions of each metabolite depend on the carbon source; the strain used or the fermentation conditions. The presence of lactic and acetic acids in fermented foods is advantageous due to their antimicrobial property, thus preventing spoilage by other microorganisms.

Significant increment in acid concentration started after 8 h of fermentation and increased linearly until 24 h, after which the production of acids was constant during the entire stationary phase. Lactic acid was the major end product of the WCJ fermentation followed by acetic acid, attaining the concentrations of 6.97, 9.69 and 12.2 g/l lactic acid, while 0.45, 0.59 and 1.15 g/l acetic acid were obtained for *L. plantarum, L. rhamnosus and L. brevis,* respectively. Absence of acetic acid results in a unilaterally sour taste with a flat and unusual aroma whereas its presence has a favourable effect on preservation. The presence of 0.51 g/l citric acid was noted at the beginning of fermentation and its concentration reduced as the fermentation progressed. At the end of 24 h fermentation only 0.16, 0.30 and 0.45 g/l citric acid was recorded for *L. rhamnosus*, *L. plantarum* and *L. brevis*, respectively.

The kinetic of lactic acid production was reasonably described by Mercier equation (Mercier et al., 1992) (Fig. 2) with a high coefficient of determination (Table 2). In the case of *L. rhamnosus*, the rate of production of lactic acid (P_r) was 0.43 h^{-1}, which was 1.72 and 2.15 times higher than that produced by *L. brevis* and *L. rhamnosus*, respectively. The numerical values of the parameter 'P_0' were found to be close to zero, which is well in agreement with the obtained results. Similar results were also obtained for *L. plantarum* and *L. brevis*, but the rate of production of lactic acid was lower (41.7 and 53.5% for *L. plantarum* and *L. brevis*) than *L.*

rhamnosus (Table 2). Some traces of citric and propionic acid was also observed although their concentration was not significant.

3.2.3 The impact of fermentation on polyphenol content

The major contribution to the AO capacity of plant foods is related to their content of polyphenols. Thus, it is important to consider the effect of fermentation on the TPC and TFC of the WCJ. Initially, the TPC of WCJ was in the range of 172.5 to 235.5 μg GAE/ml. Fermentation seemed to have a negative effect on the polyphenols content. In the case of *L. plantarum* and *L. brevis*, after 24 h of fermentation there was approx 15% reduction in TPC, while in the case of *L. rhamnosus* the loss was more prominent (24%). The TFC of the fermented WCJ at the beginning of the fermentation existed in the range of 41.9-55.6 μg QE/ml. A significant decrease in the TFC was seen as a result of LAB fermentation and approx 15.7, 21.7 and 23.9% losses in the TFC were recorded for *L. brevis*, *L. plantarum* and *L. rhamnosus* after 24 h of fermentation. These results were in agreement with the previously reported studies. Othman et al. (2009) reported a loss of 32-58% in the polyphenols during spontaneous and controlled fermentations of "Chétoui" cultivar olives. Oseni and Akindahunsi (2011) also reported a 64% reduction in the TPC during fermentation of *Jatropha curcas* and a similar trend was observed by Towo et al. (2006); these authors reported that LAB fermentation of cereal products leads to the reduction of phenolic compounds. LAB has a range of enzymes such as β-glucosidase, p-coumaric acid decarboxylase, decarboxylase which may help in degrading certain phenolic compounds. However, results obtained in the present study were encouraging as LAB fermentation retained more than 75% of the initial polyphenols in the fermented WCJ.

Degradation kinetics of the phytochemical content was modelled using zero-order (Eq. 4), first-order (Eq. 5) and second-order kinetics model (Eq. 6). Coefficient of determination (R^2) was used as statistical measures for comparison of the experimental and model simulated values. First-order kinetics model fitted the experimental data with higher R^2 value, ranging from 0.92-0.96 and 0.96-0.98 (Table 3) with a rate constant corresponding to 0.006-0.011 and 0.001-0.010 h^{-1} for TPC and TFC, respectively. The rate of TPC degradation was highest in *L. rhamnosus* fermented WCJ, which was 1.8 and 1.6 times higher than *L. plantarum* and *L. brevis*, respectively and a similar trend was observed for TFC where the rate of degradation was 1.7 and 2 times higher than *L. plantarum* and *L. brevis*, respectively.

3.2.4 The impact of fermentation on antioxidant capacity

The DPPH free radical scavenging capacity of the unfermented WCJ was in the range of 58.7-59.1 µg AscE/ml. In the case of WCJ fermented with *L. plantarum*, the loss of the DPPH radical scavenging was ≈ 13%, while in the case of *L. brevis* and *L. rhamnosus* losses were 12 and 5%, respectively, after 24 h of fermentation.

The ferric reducing AO potential of the fermented WCJ was evaluated for each strain as a function of incubation time. All the three LAB strain had similar trends during the fermentation period. The initial ferric reducing power WCJ expressed as an equivalent amount of trolox, decreased by the end of fermentation. After 24 h of fermentation, the reducing power decreased from 21.7 to 17.0 µg TE/ml when WCJ was incubated with *L. plantarum*; from 15.8 to 13.9 µg TE/ml with *L. brevis*, and from 15.0 to 13.7 µg TE/ml when inoculated with *L. rhamnosus*. AO capacity depends on the structural conformation of phenolic compounds, and a similar trend was

evident in this study. Thus, AO capacity is greatly influenced by the phenolic composition of the sample.

Degradation kinetics of the AO capacity was modelled by the application of zero-order (Eq. 4), first-order (Eq. 5) and second-order kinetics models (Eq. 6). The first-order kinetics model fitted the experimental data with a higher R^2 value, ranging from 0.87-0.98 and 0.96-0.98 for DPPH radical scavenging capacity and FRAP, respectively (Table 4) with a rate constant corresponding to 0.002-0.005 and 0.003-0.009 h^{-1} for DPPH radical scavenging capacity and FRAP, respectively. A different trend was observed in the case of AO capacity reduction, the maximum rate of reduction in DPPH scavenging capacity was evident in *L. brevis* and *L. plantarum* fermented WCJ which was 2.5 times higher than *L. rhamnosus*; however, no significant difference ($P > 0.05$) was observed between the rate of DPPH scavenging capacity reduction in *L. brevis* and *L. plantarum* fermented WCJ.

A similar pattern was observed for reduction in FRAP values, where *L. brevis* fermented WCJ showed 1.8 and 2.5 times higher rate of reduction for FRAP value. The differences in the trends between polyphenols and AO capacity could be understood by the fact that AO capacity of the food depends on the synergistic and redox interactions among the different compounds present in the vegetable. The reduction in one group of compounds may lead to the loss in functionality against certain type of free radicals without changing its functionality toward other radicals.

3.2.5 Composition of probiotic cabbage juice

The composition of food materials has an important role in maintaining prosperous health. Table 5 shows the nutritional composition of probiotic white cabbage juice. The fermented WCJ exhibited probiotic count in the range of 9.19 to 10.6 log CFU/ml. Protein concentration ranges

from 15.8 to 17.4 mg BSAE/ l while carbohydrate content in terms of glucose is 2.16 to 3.33 mg/l and fructose content ranges from 3.87 to 4.43. Among the organic acids, LA content ranged from 6.97 to 12.2 g/l while acetic acid content ranged from 0.45 to 1.15 g/l. Traces of citric acid was also noted; however, content was very low in the range of 0.16 to 0.45 g/l. Significant concentration of polyphenolic content was detected in the probiotic WCJ; which was in the range of 143.6 to 198.7 mg GAE/l.

3.2.6 Shelf life analysis

The probiotic WCJ was produced under the following conditions: an initial pH of 7.0, fermentation temperature at 37°C, inoculated with 5% (v/v) of inoculums size (≈5 log CFU/ml) and fermented for a 24 h time period. There was a complete absence of any Enterobacteria or moulds in the fermented broth during the 30 days of storage at 4ºC. LAB counts in the probiotic WCJ were 9.19, 9.47 and 10.6 log CFU/ml upon fermentation with *L. brevis*, *L. plantarum* and *L. rhamnosus*, respectively; similarly, ≈ 12.2, 6.97 and 9.69 g/l LA content were produced upon fermentation with *L. brevis*, *L. plantarum* and *L. rhamnosus*, respectively. The stability of the probiotic bacteria during storage was monitored and a slight increase (≈ 1 log CFU/ml) in the bacterial growth and lactic acid (1-2 g/l) was seen at the end of the 30 days of storage. The increase in LAB count could be due to the presence of residual glucose and fructose which was left after fermentation; thus, providing some essential growth nutrients. Pereira et al. (2011) observed in fermented cashew apple juice that up to 28 days of storage at 4°C, there was slight increment in *L. casei* count and contentious decrease in pH which could be due to acid production.

In order to exert the beneficial effects of probiotic foods, a minimum probiotic therapeutic daily dose of 8 to 9 log CFU has been proposed, which corresponds to a daily intake of 100 g of a food product containing 6 log up to 7 log CFU/g (Lee and Salminen, 1995), which this product meets. Guo et al. (2009) reported that the pH of *L. casei* fermented milk was initially 5.59 and reduced to 4.60 during storage. In addition, *L. casei* was capable in producing acid even at refrigerated temperatures which in line with that observed in this study. However, no significant changes in the TPC, TFC value and AO capacity were observed during storage.

4 Conclusion

WCJ was used as a sole source of nutrition for probiotic fermentation and was found to support prolific growth of *L. brevis*, *L. plantarum* and *L. rhamnosus*. The model equations used herein allowed accurate description of the microbial kinetics and characterization of the main products. Furthermore, the change in the response of a variable with respect to time was described. The parameters thus obtained were very useful for understanding the culture dynamics such as the rate by which a particular variable was increased/reduced or produced. All of the LAB cultures survived throughout the 30 days of refrigerated storage without substantial viability losses, phytochemical content or AO capacity loss. From the results of this study, it is concluded that probiotic WCJ could be served as a good healthy substitute of functional foods containing probiotics for vegetarians and consumers who have allergies to dairy products. Furthermore, vegetables based probiotic products would offer bioactive characteristics which are lacking in typical dairy based probiotic products. Complementary studies on the impact of the fermentation process on sensory acceptance, aroma and on the nutritional value such as vitamins and other functional properties are considered as a relevant avenue for further studies.

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Figure Captions

Fig. 1. Time course for the growth of *L. plantarum, L. rhamnosus* and *L. brevis* in WCJ

Fig. 2. Time course for the production of lactic acid by *L. plantarum, L. rhamnosus* and *L. brevis* in WCJ

Table 1. Value of parameters obtained by non-linear regression of Gompertz equation for the growth of probiotic cultures under study

	CFU/ml			
	A	μ_{max} (h^{-1})	λ (h)	R^2
L. plantarum	4.72	0.24	1.27	0.9633
	(4.13-5.32)	(0.15-0.32)	(-2.39-4.93)	
L. rhamnosus	5.88	0.36	2.81	0.9730
	(3.84-7.91)	(0.15-0.58)	(-1.47-7.08)	
L. brevis	4.31	0.33	1.99	0.9669
	(3.62-5.00)	(0.14-0.51)	(-1.75-5.74)	

Values in brackets are the confidence intervals

A: log increase in population; μ_{max}: maximum specific growth rate of cells; λ: lag phase

Table 2. Value of parameters obtained by non-linear regression of Mercier equation for lactic acid production by the probiotic cultures under study

	P_0 (g/l)	P_m (g/l)	P_r (h^{-1})	R^2
L. plantarum	0.08	8.90	0.25	0.998
	(0.04-0.12)	(8.51-9.29)	(0.22-0.28)	
L. rhamnosus	0.01	12.57	0.43	0.998
	(0.01-0.03)	(11.95-13.18)	(0.35-0.51)	
L. brevis	0.00	9.81	0.20	0.914
	(0.00-0.04)	(7.67-12.0)	(0.40-0.10)	

Values in brackets are the confidence intervals
P_o: initial lactic acid concentration; P_m: maximum concentration of lactic acid;
P_r: ratio between the initial volumetric rate of product formation (r_p) and the initial product concentration (P_o)

Table 3. Kinetics parameters for phytochemical content of WCJ during fermentation

	Zero-order kinetic			First-order kinetic			Second-order kinetic		
	C_0	k (h^{-1})	R^2	C_0	k (h^{-1})	R^2	C_0	k (h^{-1})	R^2
L. plantarum									
TPC	170.7	0.910	0.92	171.6	0.006	0.93	172.5	0.0001	0.91
TFC	42.6	0.352	0.97	42.9	0.001	0.98	42.7	0.0003	0.97
L. rhamnosus									
TPC	188.5	1.744	0.92	190.9	0.011	0.94	190.2	0.0001	0.94
TFC	40.7	0.336	0.95	41.1	0.010	0.97	41.0	0.0003	0.96
L. brevis									
TPC	236.8	1.429	0.96	238.2	0.007	0.97	238.8	0.0001	0.94
TFC	56.1	0.400	0.96	56.2	0.005	0.97	56.8	0.0002	0.94

C_0 Model estimated value, k reaction rate constant

Table 4. Kinetics parameters for antioxidant capacity of WCJ during fermentation

	Zero-order kinetic			First-order kinetic			Second-order kinetic		
	C_0	k (h^{-1})	R^2	C_0	k (h^{-1})	R^2	C_0	k (h^{-1})	R^2
L. plantarum									
DPPH	57.7	0.288	97.5	57.9	0.005	97.6	58.1	0.0001	97.2
FRAP	15.7	0.069	96.6	15.7	0.005	96.9	15.8	0.003	95.3
L. rhamnosus									
DPPH	59.2	0.110	84.4	59.1	0.002	87.4	59.3	0.00003	84.1
FRAP	15.0	0.041	97.6	15.0	0.003	97.6	15.0	0.0002	95.5
L. brevis									
DPPH	57.6	0.251	86.3	57.7	0.005	87.1	56.8	0.0001	82.2
FRAP	21.0	0.166	95.1	21.2	0.009	96.2	21.2	0.0005	96.4

C_0 Model estimated value, k reaction rate constant

Table 5. Nutritional composition of probiotic cabbage juice

	LAB strain used for juice preparation		
	L. brevis	*L. plantarum*	*L. rhamnosus*
Probiotic count (log CFU/ml)	9.19 ± 0.24	9.47 ± 0.15	10.6 ± 0.23
Protein content (mg BSAE/l)	16.4 ± 0.73	15.8 ± 1.46	17.4 ± 0.30
Carbohydrate content			
Glucose (mg/l)	3.33 ± 0.29	2.31 ± 0.17	2.16 ± 0.22
Fructose (mg/l)	3.87 ± 0.26	4.41 ± 0.15	4.43 ± 0.13
Organic acid content			
Lactic acid (g/l)	12.2 ± 0.18	6.97 ± 0.29	9.69 ± 0.01
Acetic acid (g/l)	1.15 ± 0.11	0.45 ± 0.07	0.59 ± 0.12
Citric acid (g/l)	0.16 ± 0.01	0.30 ± 0.05	0.45 ± 0.09
Polyphenolic content			
TPC (mg GAE/l)	198.7 ± 2.89	148.6 ± 1.32	143.6 ± 1.75
TFC (mg QE/l)	46.9 ± 1.24	35.3 ± 1.54	31.9 ± 1.02
Antioxidant capacity			
FRAP value (mg TE/l)	13.9 ± 0.02	17.0 ± 0.12	13.7 ± 0.17
DPPH RSA (mg AAE/l)	51.9 ± 0.45	51.1 ± 0.79	56.1 ± 0.32

LAB, Lactic acid bacteria; BSA, Bovine serum albumin; TPC, Total phenolic content; GAE, Gallic acid equivalent; TFC, Total flavonoid content; QE, Quercetin equivalent; TE, Trolox equivalent; AAE, Ascorbic acid equivalent; FRAP, Ferric reducing antioxidant potential; RSA, Radical scavenging capacity

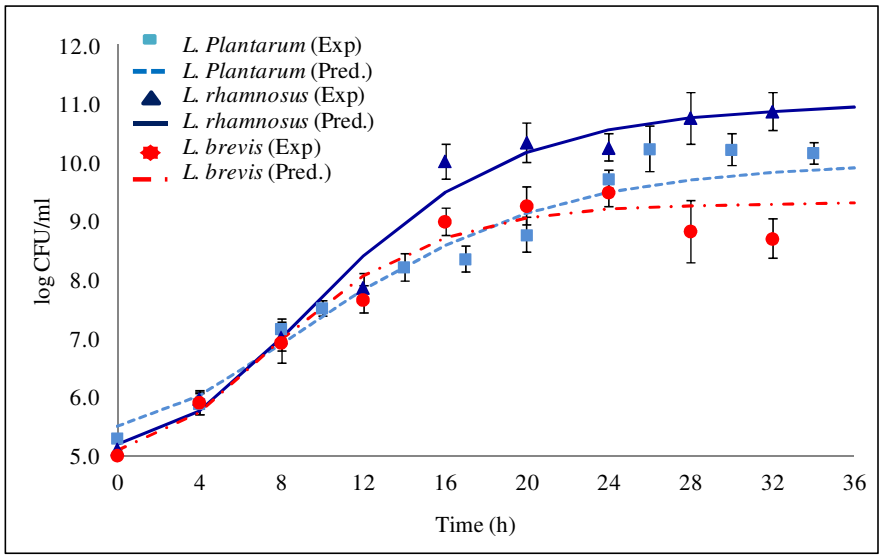

Fig. 1. Time course for the growth of *L. plantarum, L. rhamnosus* and *L. brevis* in WCJ

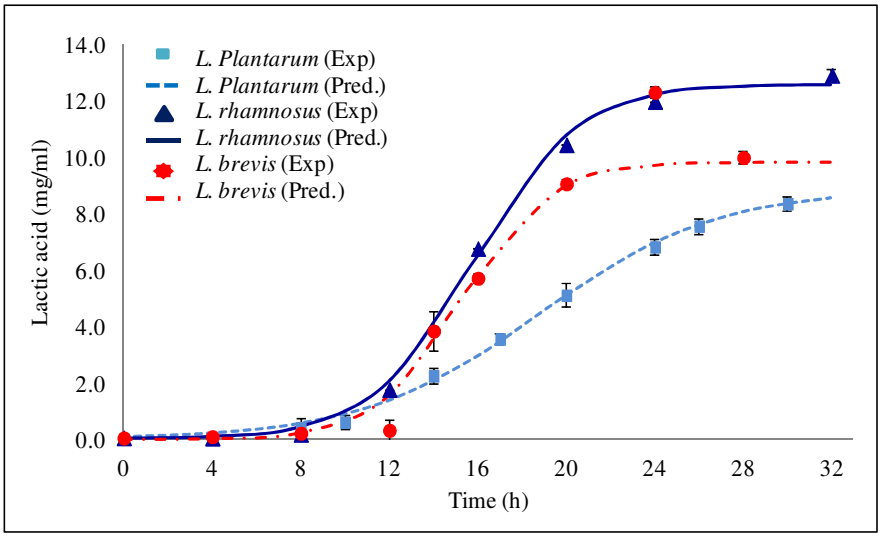

Fig. 2. Time course for the production of lactic acid by *L. plantarum, L. rhamnosus* and *L. brevis* in WCJ