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Research Article

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Stabilizing Effect of Vitamin C on Sulforaphane Level in A Controlled-Fermented Broccoli Sprouts

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Abstract

This study investigates the sulforaphane levels in broccoli sprouts samples produced under a controlled-lactic acid fermentation. Such process includes combining broccoli sprouts, a cruciferous vegetable, with twelve selected strains of lactic acid bacteria: Lactobacilli, Streptococci, and Bifidobacteria to form a mixture that undergoes lactic acid fermentation, transforms glucosinolate within the cruciferous vegetable to sulforaphane, and yield a fermented mixture that contains sulforaphane, and subsequently from that fermented mixture manufacturing nutritional food item that can be ingestable and sustainable for humans. pH decrease within the lag phase is the critical and decisive moment for the end product sulforaphane stability.

Purpose

Various sulforaphane powders, pills, and probiotics are commercially available. However, sulforaphane powder mixed in water is only stable over a short period and must be consumed as soon as possible, often within thirty minutes of mixing. Accordingly, there is a desire for a convenient and durable source of sulforaphane capable of delivering the nutritional benefits of sulforaphane, aside from the direct consumption of fresh broccoli sprouts. This study provides a process that answers that question and addresses the sulforaphane availability in post-oro-maxillofacial surgery patients.

Methods

Lactic acid fermentation of broccoli sprouts triggered by twelve homo- and heterofermentative Lactobacilli strains. Early addition of the vitamin C at lag phase; Cell cultures grow in MRS-broth then on MRS-agar followed by a sulforaphane evaluation using HPLC method.

Results

A mixture of twelve selected hetero- and homofermentative Lactobacilli strains employed during controlled-lactic acid fermentation enable the transformation of glucosinolate from broccoli sprouts to a stable sulforaphane level of 0.130 mg/g with a sustainable nutritional value. The mixture's Lactic acid bacteria (LAB) count remained high (2500000 cells/g), and we detected no coliform bacteria. The early addition of vitamin C is essential to stabilizing of sulforaphane content of this study.

Conclusion

This study yields a nutritional and microbiological stable sulforaphane-containing product ingestible by humans whose sulforaphane levels survived the processing mechanism and can sustain daily dietary requirements in vitamins, minerals, proteins, and carbohydrates.

Keywords: Sulforaphane Level, Broccoli Sprouts, Lactic Acid Bacteria, Sulforaphane Bioavailability

Abbreviations

LAB: Lactic acid bacteria
HPLC: High-performance liquid chromatography
MRS-agar (broth): de Man, Rogosa and Sharp-agar
B: Bifidobacterium
ITCs: Isothiocyanates
HoLAB: Homofermentative Lactic Acid Bacteria
HeLAB: Heterofermentative Lactic Acid Bacteria

1. Introduction

Sulforaphane is known as a long-lasting antioxidant, who's anti-cancerogenic effects were confirmed by in-vitro studies conducted by John Hopkins University School of Medicine [1-11] and confirmed by multiple publications [12-14]. Sulforaphane is obtained from cruciferous vegetables (the family of Brassicaceae, formerly Cruciferae), non-limiting examples of which include brussels sprouts, cabbages, cauliflower, broccoli (particularly broccoli sprouts). A series of encyclopedias have extensively reported on cruciferous vegetable origin, genetics, and chemistry, especially how glucoraphanin (a glucosinolate) reacts with myrosinase (an enzyme present in the plant cell) to form sulforaphane [15-19,11]. The protective effect of these micronutrients is due to the inhibition of phase-I carcinogen-bioactivating enzymes and induction of phase-II detoxifying enzymes [5-10]. The protective effect of glucoraphanin is thought to be due to sulforaphane, an isothiocyanate metabolite formed from glucoraphanin by the enzyme myrosinase [3-5,20,21]. The reaction between myrosinase and glucosinolate occurs in the gut after glucosinolate has been made available by chewing such that sulforaphane is produced after the swallowing event occurs.

Storage, processing, and cooking can change ITCs formation from glucosinolate and affect the cruciferous vegetables' anticancer activity [12]. Intake of raw cruciferous vegetables provides two to nine times the number of ITCs in humans compared with similar intakes of their cooked counterparts due to heat-inactivating myrosinase, which reduces sulforaphane formation [13,14,22,23].

Human intestinal microflora also possesses myrosinase activity, able to partially hydrolyze ingested glucosinolates which is an advantage to this study with the use of Lactobacilli strains. Gut microflora is mainly responsible for converting ingested glucosinolates to their cognate isothiocyanates [14].

Despite their nutritional and health benefits, including being a source of sulforaphane, broccoli sprouts have certain disadvantages that have limited their use. For instance, broccoli sprouts tend to spoil quickly, and contamination with coliform bacteria is a well-known safety issue, resulting in unwanted additives often accompanying broccoli sprouts. Several studies have elucidated microbial fermentation's positive impact on product shelf life and the protective properties of lactic acid fermentation. Balows et al., 1991 also highlighted the prokaryotic ecophysiology, characterization, and applications [24-46].

Apart from providing an edible fermented new product capable of delivering sulforaphane's nutritional benefits (figure 1), this study offers ways to avoid drawbacks associated with the consumption of cruciferous vegetables, especially broccoli sprouts.

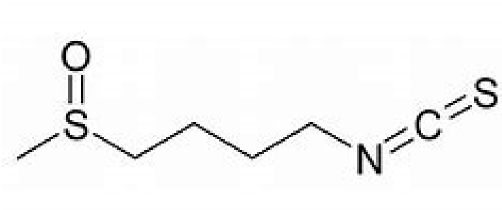

Figure 1: Sulforaphane chemical formula

2. Materials and Methods

Processing Brief Description

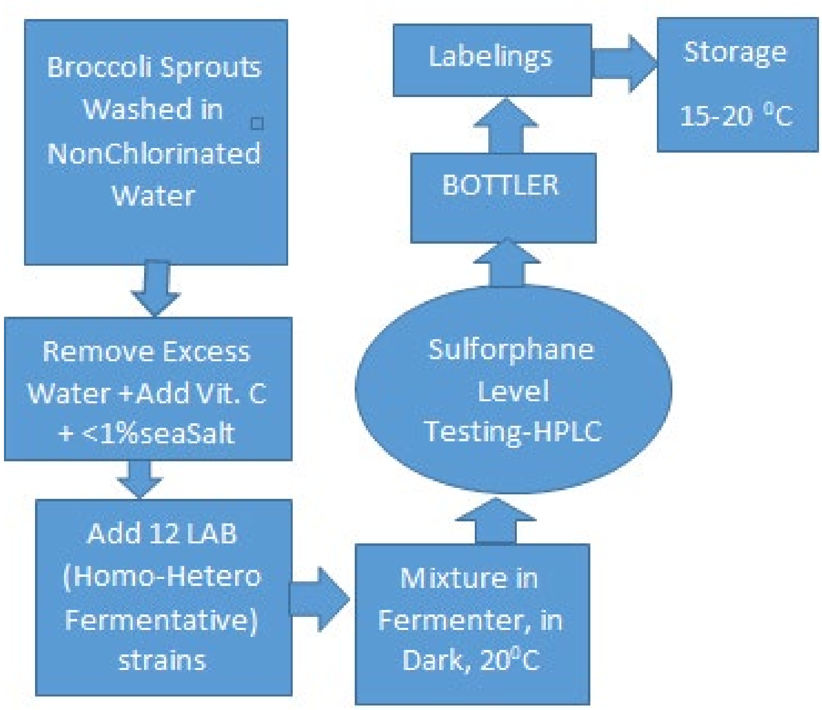

Figure 2: represents a flow diagram of a process for probiotics broccoli sprouts.

As outlined in figure 2, we sanitize broccoli seeds before being planted to grow broccoli sprouts. As used herein, broccoli sprouts will refer to new growth from seed germination, a plant that is a few (e.g., three to four) days old. After harvest, the sprouts are washed in purified water and dried to reduce the presence of bacteria on the sprouts. The sprouts are then combined with LAB strains and ingredients (<1% NaCl) which trigger a lactic acid fermentation that transforms glucosinolate contained in the sprouts into sulforaphane-containing product. Fermentation produces sulforaphane without requiring chewing or physically breaking up the sprouts to make the glucosinolate available and without the requirement for contacting and reacting the sprouts with myrosinase. The broccoli sprouts are combined with twelve strains of Lactobacilli, which are part of the lactic acid bacteria (LAB) group and known as probiotics. The novelty of this study is the addition of vitamin C at the earlier stage (5-10 minutes) during LAB growth lag phase.

The fermentation has been successfully conducted in fermenters under anaerobic and dark environments (i.e., shielded from and without natural and artificial lighting) at room temperatures of about 20^{o}C.

3. Lab and Methods

In this particular study, organic broccoli sprouts germinated over three days were washed three times in non-chlorinated purified water, after which the following twelve selected strains of lactobacilli were added: *B. bifidum, B. lactis, B. infantis, B. longum, L. acidophilus, L. brevis, L. bulgaricus, L. paracasei, L. plantarum, L. rhamnosus, L. salvaricus, and Streptococcus thermophilis* from “Nature” were used as starter cultures.

These chosen strains are known for their physiological benefits and antimicrobial capabilities. Sea salt <1% and, especially, vitamin C added to this mixture create an early acidic pH suitable for lactic acid fermentation/Reference samples without vitamin C, conducted anaerobically in a dark environment at about 20^{0}C for seven days in handmade 3-layered wood-ceramic-glass fermenter (figure 3).

LAB-Analyses: By CMMEF, 4th Ed.; MRS-Agar (broth); E. coli & Coliform (by Petrifilm-AOAC 991.14)

Sulforaphane Content Evaluation: By Solid Phase Extraction (SPE)-High-Performance Liquid Chromatography (HPLC) by Han D.et al.

Minerals and Vitamins Evaluation: Calcium by ICP (AOAC 984.27); Iron by ICP; Vitamin A-Beta Carotene (Analyst 1984 109:48), Vitamin C (HPLC)- 53 mg Vitamin C in 113 g sprouts

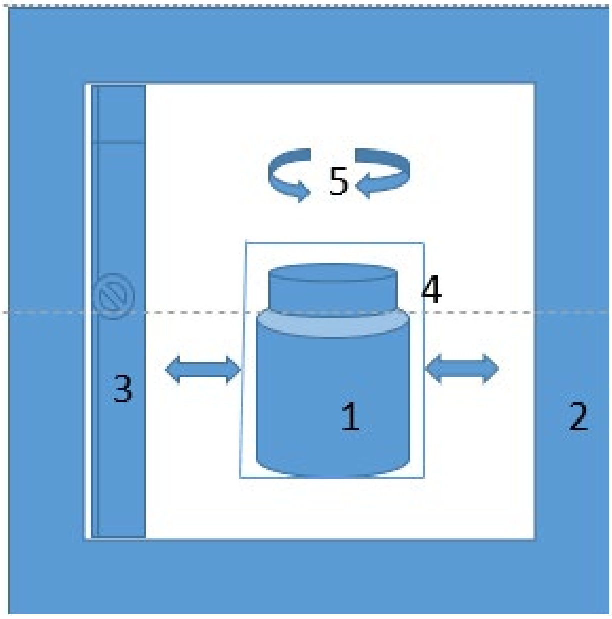

Figure 3: Three- layered Fermenter Technology (external layer:woody#2, middle layer: ceramic#4, internal layer: glass#1;door opening#3; reduced air circulation #5)

4. Results

The process from this study yields a microbiologically safe and durable natural formula that contains sulforaphane before being ingested by an individual and is capable of delivering benefits to the individual when ingested.

After seven days at these conditions described above, the mixture was found to be fermented, and the mixture tested positive (0.130 mg/g) for sulforaphane. The Lactic acid bacteria (LAB) count of the mixture remained high (2500000 cells/g), pH-decrease from 5.0 to 3.9 (Figure 4) and no coliform bacteria was detected. Following storage of the fermented mixture in a refrigerator over a two-month period, coliform bacteria were not detected either. After six months of storage in a refrigerator, the amount of sulforaphane dropped to 50% (0.0651 mg/g), which is a good result given the instability of that phytochemical.

As such, this study processing method results in a broccoli sprouts mixture containing sulforaphane-probiotics with notable nutritional benefits, enriched with vitamins and minerals, which contribute to answering world challenges regarding food scarcity and sustainability.

Total fat	12.7g/100g
Saturated fat	0.12 g/100g
Trans fat	0.12g/100g
Sodium	568mg/100g
Total carbohydrate	5.2g/100g
Dietary fibers	2.11g/100g
Sugars	1.6g/100g
Protein (f=6.25)	2.88g/100g
Calcium	38.3 mg/100g
Iron	0.77mg/100g
Vitamin A	662 IU/100g
Vitamin C	30.11 mg/100g
Sulforaphane	0.130 mg/g (triplicate samples)
LAB	2500000 cells/g
E.coli/Coliform	Not detected

Table 1: Nutritional and microbiological evaluation of fermented broccoli sprouts

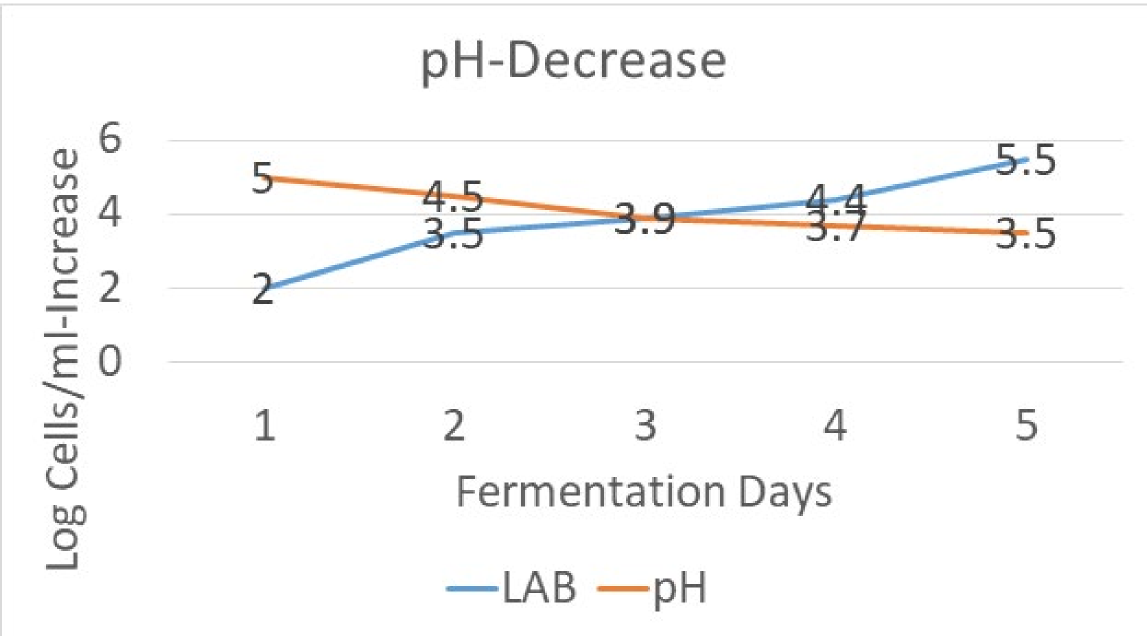

Figure 4: pH-Decrease within lag-phase corrreponding to LAB Increase (log cell count/ml)

5. Conclusion and Discussion

The broccoli sprouts combined with twelve specific LAB, as described in this study yielding to the current new product, have been found to trigger a lactic acid fermentation that transforms glucosinolate contained in the sprouts to sulforaphane. Selected Lactobacilli (HeLAB and HoLAB) strains employed by the process in this study yield a sulforaphane-containing product and enhance the stable sulforaphane levels [13,14,22,23].

Lactic acid Fermentation from this study produces sulforaphane without requiring chewing or otherwise physically breaking up the sprouts to make the glucosinolate available and without the requirement for contacting and reacting the sprouts with myrosinase outside the human GI (Gastrointestinal)-tract.

The end product from this study can remain in the form of a consumer food item that can be directly ingested by an individual.

In addition to consumer food items, this study fermented products may be processed and packaged for use by medical personnel and nutritionists, in a variety of industries and practices, for example, as a pill, powder, for use in pharmaceuticals, nutraceuticals, preventive medicines, food processors, etc. This fermented product can particularly benefit individuals with digestive or other specific health issues. The shelf-life of this fermented product is up to two mounts between 10ºC to 15ºC temperature.

While this study has focused on twelve LAB strains, new studies could use other forms of the mixture; for example, though twelve specific strains of Lactobacillus were used in this study, hypothetically, it may be possible to omit or add some of the used strains of lactobacilli, and yet yield a fermentation that may produce a suitable fermented mixture that contains a sustainable amount of sulforaphane. In addition, it is foreseeable that multiple cruciferous vegetables could also be combined and fermented. We should keep in mind that the processing of Brassica vegetables has negatively impacted the contents of glucosinolates and has reduced their health-protective capabilities [12]. The other question remains on the impact of the ratio of homo- versus heterofermentative strains on the stabilization of sulforaphane level. On the contrary, it is fair to affirm that this study's vitamin C addition at the lag phase in three-layered fermenter is the turning and essential point of this research paper's contribution. It brings some hope regarding the end product's sulforaphane levels and stability, which may contribute positively to food sustainability challenges and opportunities. You can grow it in your environmentally friendly backyard, and its scope provides countless applications [5-10]. Even though the daily value for sulforaphane is still unknown, its amount in this paper's results is encouraging for consumers with oro-maxillofacial chewing issues. One could investigate monthly radiological and imaging evaluations of sulforaphane using this study's new processing method.

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Author & Co-Author Contributions

The manuscript was conceived, conducted and written by the author and reviewed by the co-author. Both agreed on the last draft.

Conflicts of Interest

The author declares no conflict of interest.

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