ORIGINAL ARTICLE

APPLICATION OF A NOVEL STARTER CULTURE FOR FERMENTED VEGETABLES AND FRUITS

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ABSTRACT

Background: The great challenge for the food industry is to produce suitable strain cultures for fermented products. Controlled fermentations using starter culture have allowed better control of the fermentation process and has prevented the spoilage of non-desirable microorganisms. **Objectives:** The aim of this work was the look of the application of a suitable selected starter with a minimum of microorganisms for fermented lemons, carrots at this stage and other products for future studies. **Methods:** *Lactobacillus amylovorus* and *Candida guillermondii* selected in previous studies were applied as a starter culture. Selection of strains as inoculum for fermentation of the plant material focused on rapid acidification, growth in low pH and tolerance to the high salt concentrations (10% to 15%of salt). Controlled fermentations were conducted at 10% of salt during 68 days. Cell counts (yeasts, lactic acid bacteria, TAMF, Coliforms), contents of organic acids, pH and ambient temperature were measured. **Results:** The content of organic acids increased to reach 1.4% for carrots and 2.3% for lemons at the end of fermentation. PH dropped during fermentation from 7,00 to 2.7 after 21 days for carrots and to 2 after 26 days for lemons. **Conclusions:** *Candida guillermondii* and *Lactobacillus amylovorus* used as a starter culture for the first time for lemons and carrots, allowed us to obtain stable products for 6 months. Homogeneous and satisfactory final hygienic quality was obtained. Coliforms were absent after seven days of fermentation, mesophilic flora strains were present at a low concentration at the end of fermentation. Final pH values, acid development and hygienic quality support the potential of*Lactobacillus amylovorus and Candida guillermondii* as a starter culture. The results may also lead toindustrial applications and the uses of the same starter culture for eventual products.

Keywords: *starter culture, vegetables and fruits fermentation, carrots, lemons, L. amylovorus, C.guillermondi.*

1. INTRODUCTION

As world population rises, lactic acid fermentation is expected to play an important role in preserving fresh vegetables, fruits and other food items [1]. Fermented fruit or vegetable products are part of various diets worldwide.In fact, nutritional recommendations emphasize fruit and vegetable consumption. This is related to health-benefits associated with bioactive nutritive molecules. In fruits and vegetables, molecules of nutritional interest are fibers, vitamins, minerals, phenolic compounds, bioactive peptides. Fermentation is a natural process that is probably with drying and refrigeration the best mode of preservation especially in countries without a developed industrial structure [2]. Fermentation is a process which involves slow decomposition of organic substances, induced by enzymes or microorganisms, which basically convert carbohydrates into organic acids. More recently, the consumption of fermented foods containing live microorganisms has emerged as an important dietary strategy for improving human health [3, 4, 5]. To improve fermentation and high quality and safety of the final products, the use of starter cultures have been explored in vegetable fermentation. Starter cultures are an essential component of approximately all commercially produced fermented foods [6]. The importance of fermentation is underlined by the large spectrum of foods marketed both in developing and industrialized countries, not only for the benefit of preservation and safety, but also for their sensory attributes [7]. Some fermented food products have a global distribution, whilst others are restricted to particular human cultures. Basically, to pilot a fermentation process some producers prefer to rest the quality of their products on the performance of natural microorganisms, whereas others prefer to use commercial starter cultures to ensure a predictable process and avoid the production of undesired metabolites [8]. Extrapolating the conclusions drawn from our previous studies [9, 10, 11, 12, 13, 14] and after a successful test for fermented olives [13, 14]. The purpose of this study was to apply a unique starter culture for lemons and carrots .We tried to apply a mixture of strains containing *candida guillermondii* and *lactobacillus amylovorus* as a starter culture at 10% of salt, in the look for the standardization and the application of the same starter culture for other plant material in the future. This is the report of a successful use of a defined, mixed starter culture in such fermentation.

2. MATERIALS AND METHODS

2.1 Protocol of fermentation:

Lemons and carrots collected from local markets were simultaneously inoculated with a mixture including a member of yeasts (*candida guillermondi*) and the lactic acid bacterium (*Lactobacillus amylovorus*) at a rate of 1.5%. A concentration of 3 g/L of glucose is added to the brine at 10% of salt. All the fermenters were maintained at ambient temperature.

2.2Sampling and monitoring the fermentation:

Samples were collected periodically. Parameters measured were: pH, temperature and acidity. The pH measurements of brines were obtained with a Crison pH meter model 2001(Crison Instruments, Barcelona, Spain), calibrated with two standard solutions buffered at pH=4.00 and7.02.The titratable acidity is expressed as % of lactic acid and is determined in 10 ml of brines with (N/9) NaOH using phenolphthalein as indicator. The ambient temperature is measured using a standard thermometer. Bacterial growth (CFU per ml) was quantified through plating of 10-fold serial dilutions of the samples in distilled water and the dilutions were plated in duplicate onto appropriate media at the appropriate temperature. The enumeration of the Total Aerobic Mesophilic Flora (TAMF) was performed on Plate Count Agar (PCA) at 30 °C for 48 h, coliforms on Deoxycholate Agar medium (DCL) [15] at 37 °C for18–24 h, lactic acid bacteria on Man Rogosa and Sharp Agar medium (MRS) [16] and yeasts on potato dextrose agar medium (PDA) [17].

3. RESULTS

Table1: presents the enumeration of bacterial and yeasts charges of controlled fermentation of lemons.

Time of fermentation (days)	TAMF (cfu/ml)	coliforms (cfu/ml)	LAB (cfu/ml)	YEASTS (cfu/ml)
3	5.10^{4}	0	$5,9.10^{4}$	$7,9.10^{4}$
7	$4.4.10^{4}$	0	$1,9.10^{4}$	$8,4.10^{4}$
10	$2,8.10^{4}$	0	3.10^{4}	$2,9.10^{4}$
15	1.10^{4}	0	2.10^{3}	$2,3.10^{3}$
17	3.10^{4}	0	6.10^{2}	$1,6.10^{5}$
22	$1,1.10^{4}$	0	0	7.10^{3}
25	$6,9.10^{3}$	0	0	$2,9.10^{5}$
31	$2,2.10^{3}$	0	0	3.10^{5}
38	$6,5.10^{3}$	0	0	$1,5.10^{4}$
45	$3,7.10^{3}$	0	0	$2,73.10^{4}$
55	$03,2.10^{3}$	0	0	3.10^{4}

Table 2: shows the stability of the physicochemicals parameters during the controlled fermentation of lemons

Time	Ph	Acidity	temperature
T0	6.50	0	25 °C
1 MONTH	2.03	2	25°C
3 MONTHS	2.06	2.3	24°C
6 MONTHS	2.06	2.3	23°C

Table 3: shows the stability of the physicochemicals parameters during the controlled fermentation of lemons.

Time	Ph	Acidity	temperature
T0	6.70	0	25 °C
1 MONTH	2.43	1.5	25°C
3 MONTHS	2.76	1.4	24°C
6 MONTHS	2.76	1.4	23°C

Table4: presents the enumeration of bacterial and yeasts charges of controlled fermentation of carrots.

Time of fermentation	TAMF CFU/ML	COLIFORMS CFU/ML	LAB CFU/ML	YEASTS CFU/ML
2	0,53 10^5	0	1.10^4	2,3.10^5
6	2,95 10^4	0 0	5.10^4	7,1.10^5
9	1,43.10^4	0	2,8.10	2,13.10^5
14	2.10^4	0 0	2,6.10^3	3,5.10^5
16	1,12.10^4	0	2.10^2	1,36.10^5
21	1,31.10^4	0 0	0	2,2.10^5
23	7,81.10^3	0	0	9,6.10^5
29	0,57.10^3	0 0	0	23,4.10^5
37	18.10^3	0	0	22.10^5
45	26.10 ^{3}	0 0	0	48 10^5
55	21.10 ^{3}	0	0	42 10^5

4. DISCUSSION

Fruits and vegetables are easily perishable commodities due to their high water activity and nutritive values. Lactic acid fermentation increases their shelf life and also enhances several beneficial properties, including nutritive value, flavours and reduces toxicity [19, 20]. The acidification of food by lactic acid inhibits manypathogenic strains and the decrease of pH is the most important criterion for selection of starter cultures [21, 22]. It is a crucial factor for reduction of the growth of accidental microflora. The tables 2 and 3 revealed a decline in pH to 2.06 for lemons and 2.76 for carrots due to the metabolism of sugars contained in the medium by lactic acid bacteria. After 1 month the pH remain stable and the products are stable for 6 months at ambient temperature. Coliforms are absent in the two essays. The levels of yeasts and lactic acid bacteria developed during the controlled fermentation were quantitatively examined.Mesophilic flora strains were present at a low concentration at the end of fermentation: 3.210^3 ufc/ml for fermented lemons 21 10^3 ufc/ml for fermented carrots. Yeasts were present at a rate of 42.10^5ufc/ml for fermented carrots, 3.10^4ufc/ml for fermented lemons. For lactic acid bacteria the results of the enumeration on MRS medium at the end of fermentation were respectively 6.10^2ufc/ml for lemons and 2.10^3ufc/ml for carrots.The final lactic acid concentration conforms to the hygienic standards [23, 24]. In general, lactic acid bacteria (LAB) from several genera, including *Lactobacillus, Streptococcus*, and *Leuconostoc* are predominant in fermented foods, but other bacteria as well as yeast and fungi also contribute to food fermentations [25, 26, 27]. Lactobacilli produce a wide range of inhibitory compounds such as organic acids (lactic acid), hydrogen peroxide, diacetyl, bacteriocins and compete with other microorganisms by nutrient depletion [28]. Lactobacilli are also associated with traditional fermented products and thus have the Generally Recognized as Safe (GRAS) status granted by the US Food and Drug Administration (USFDA) [29]. Lactic acid bacteria are generally fastidious on artificial media, but they grow readily in most food substrates and lower the pH rapidly to a point where competing organisms are no longer able to grow [30, 31, 32, 33, 34, 35]. In fact, in previous studies, exploring spontaneous fermentations of some plant material lead to the isolation of *Lactobacillus amylovorus* and *Candida guillermondii* with relevant technological properties. This strain meets most of the criteria for industrial application. The ability to produce acids, to cope with high NaCl concentration 15% and 10%, acidic pH and high temperatures are the most criteria for the selection of *lactobacillus amylovorus and Candida guillermondii* as a starter culture [8, 9, 10, 11, 12, 13, 14, 15]. It is noted that the lactic bacteria ensures the beginning of fermentation and it is relayed after by the yeast (table 1 and 3). Indeed, *Leuconostocs* and lactic streptococci generally lower the pH to about 4.0 to 4.5, and some of the lactobacilli and pediococci to about pH 3.5, before inhibiting their own growth [36]. Mainly in food industry the Food and Drug Administration (FDA) has permitted *C. guilliermondii* (ATCC 20474) and *Candida lipolytica* to be used in food for human consumption as secondary direct food additives in the production of citric acid; *Candida guilliermondii* has been studied over the last 40 years due to its biotechnological interest, biological control potential and clinical importance [30]. In literature**,** Yeasts of this genus (*Candida guillermondii*) used as a starter culture in this study are involved in important human opportunistic infections and they have many industrial applications**.***Candida guilliermondii* has been the sixth frequently isolated Candida species : it's an emerging pathogen in Latin America that rarely causes invasive candida infections, opportunistic Fungal Pathogen with Decreased Susceptibility to Fluconazole. Furthermore, Luanne augusto and *al.,* (2003) proposed that *C guilliermondii* must be reclassified as *C. tropicalis*, PCRq and BLAST analysis revealed strong identity with the corresponding sequences from *Candida tropicalis* [32]. Feng X and *al.*, (2014) showed that some ofemerging pathogens *Candida palmioleophila*, *Candida fermentati*, and *Debaryomyces nepalensis* are often misidentified

as *Candida guilliermondii* or *Candida famata* in the clinical laboratory [37]. In fact extrapolating the results and bibliographic data, this present study developed an effective starter culture which enhances the hygiene, sensory and shelf life properties of the products.

4. CONCLUSION

Actually the modern starter culture industry provides cultures for nearly every type of fermented food and beverage. The results of the monitoring showed a significant decrease in pH. *Lactobacillus amylovorus* in mixture with *Candida guillermondii* initiated rapid acidification. The results clearly show the performance and the usefulness of the new starter culture used for the first time to control the lemons and carrots fermentations and may lead to industrial applications and future tests for other products.

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