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4.3.4. Технологии, машины и оборудование для лесного хозяйства и переработки древесины
4.3.5. Биотехнология продуктов питания и биологически активных веществ

ВЛИЯНИЕ ЭКСТРАКТОВ ДИОСКОРЕИ КЛУБНЕНОСНОЙ И КОЛЕУСА ПРОТИВОДИЗЕНТЕРИЙНОГО НА ЖИЗНЕСПОСОБНОСТЬ BIFIDOBACTERIUM LACTIS JYBR-190 И LACTOBACILLUS CASEI JYLC-374

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Настоящее исследование проанализировало потенциал экстрактов Dioscorea bulbifera (DB) и

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4.3.4. Technologies, machinery and equipment for forestry and wood processing
4.3.5. Biotechnology of food and biologically active substances

EFFECT OF DIOSCOREA *BULBIFERA* AND *COLEUS DYSENTERICUS* EXTRACTS ON THE VIABILITY OF *BIFIDOBACTERIUM* LACTIS JYBR-190 AND *LACTOBACILLUS CASEI* JYLC-374

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The present study analyzed the potential of *Dioscorea bulbifera* (DB) and *Coleus dysentericus* (CD) extracts

Coleus dysentericus (CD) в отношении жизнеспособности Bifidobacterium lactis JYBR-190 (BIL) и Lactobacillus casei JYLC-374 (LAC). Химический анализ этих субстратов выявил высокие концентрации Ca - 1240 ± 41 мг/кг, Mg - 3332 ± 64 мг/кг, Na - 58,5 ± 2 мг/кг, K - 18301 ± 50mg/Kg и Fe - 510 ± 86 мг/кг для D. bulbifera и Ca - 437 ± 20 мг/кг, Mg - 2048 ± 142 мг/кг, Na - 109 ± 2 мг/кг, K - 7182 ± 500 мг/кг и Fe - 137 ± 16mg/Kg для C. dysentericus; но также и в клетчатке, с концентрациями 5,95 ± 0,1% для D. bulbifera и 1,53 ± 0.1% для C. dysentericus соответственно. B. lactis JYBR-190 и L. casei JYLC-374, культивируемые в экстрактах этих двух субстратов, показали легкий рост вплоть до превышения приемлемого порога ВОЗ для пробиотически назначенных продуктов. Быстрый рост наблюдается при разведениях 10^{-3} и 10^{-4}, и по мере увеличения концентрации экстракта Bifidobacterium lactis JYBR-190 и Lactobacillus casei JYLC-374 расти более легко. Их глобальное среднее значение (Mglobal) роста продемонстрировало действительно конкурентоспособность между двумя субстратами с более положительным эффектом, связанным с Dioscorea bulbifera, чем Coleus dysentericus. Модельный анализ показал, что симбиотическое культивирование обоих микроорганизмов в обоих субстратах еще больше ускорило их развитие, облегчая снижение разбавления для достижения идеальных условий. Сравнительный статистический анализ показал, что термическое изменение субстратов вредно для развития обоих типов микроорганизмов. Исследования показывают, что холодное хранение продукта ослабляет стабильность микроорганизмов продукта. От 3 до 30 дней микроорганизмы быстро разрушаются в контрольном образце и частично в исследованных субстратах. Однако стабилизирующие свойства изученных субстратов все же имеют свои пределы: через 25 дней они исчезают по мере падения содержания микроорганизмов ниже допустимого ВОЗ порога. Но криозащитное и стабилизирующее действие исследуемого субстрата оценивали между 20 и 50% для обоих микроорганизмов

on the viability of *Bifidobacterium lactis* JYBR-190 (BIL) and *Lactobacillus casei* JYLC-374 (LAC). Chemical analysis of these substrates revealed high concentrations of Ca - 1240±41mg/Kg, Mg - 3332±642 mg/Kg, Na - 58.5 ±2 mg/Kg, K - 18301 ± 50mg/Kg and Fe - 510 ±86mg/Kg for *D. bulbifera* and Ca - 437±200 mg/Kg, Mg - 2048 ±142mg/Kg, Na - 109 ±2mg/Kg, K - 7182 ± 500 mg/Kg and Fe - 137 ± 186mg/Kg for *C. dysentericus* ; but also in fiber, with concentrations of 5.95±0.011% for *D. bulbifera* and 1.53±0.004% for *C. dysentericus* respectively. *B. lactis* JYBR-190 and *L. casei* JYLC-374, cultivated in extracts of these two substrates, showed easy growth up to the point of exceeding the WHO acceptable threshold for probiotically-appointed products. Rapid growth is observed at dilutions of 10^{-3} and 10^{-4}, and as the extract concentration increases, Bifidobacterium lactis JYBR-190 and Lactobacillus casei JYLC-374 grow more readily. Their global average (Mglobal) of growing demonstrated a really competitiveness between the two substrate with a more positive effect related to *Dioscorea bulbifera than Coleus dysentericus.* A model-based analysis showed that symbiotic cultivation of both microorganisms in both substrates further enhanced their development, making it easier to lower the dilution to reach ideal conditions. Comparative statistical analysis showed that thermal alteration of the substrates was detrimental to the development of both types of microorganisms. Studies show that cold storage of the product weakens the stability of the product's microorganisms. From 3 to 30 days, microorganisms deteriorate rapidly in the control sample and partially in the substrates studied. However, the stabilizing properties of the substrates studied still have their limits: after 25 days, they disappear as the microorganism content falls below the WHO acceptable threshold. But the cryo-protector and the stabilizer effect of the studied substrate was evaluated between 20 to 50 % for the both microorganisms

Ключевые слова: ТРАДИЦИОННЫЕ КУЛЬТУРЫ, ПРОБИОТИКИ, ПРЕБИОТИКИ, МИНЕРАЛЬНЫЕ ЭЛЕМЕНТЫ, ВОЛОКНА, DIOSCOREA BULBIFERA, COLEUS DYSENTERICUS, BIFIDOBACTERIUM LACTIS JYBR-190, LACTOBACILLUS CASEI JYLC-374

Keywords: TRADITIONAL CULTURES, PROBIOTICS, PREBIOTICS, MINERALS ELEMENTS, FIBERS, *DIOSCOREA BULBIFERA*, *COLEUS DYSENTERICUS*, *BIFIDOBACTERIUM* LACTIS JYBR-190, *LACTOBACILLUS CASEI* JYLC-374

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1. Introduction.

Probiotic products have long existed throughout the world and were generally initiated to supplement certain deficiencies linked to the flora of the digestive tract.

According to the authors, several technological arrangements have been made to increase the functional efficacy of these products: namely, the use of plant extracts such as inulin (JOUZIER & BERKÉ, 2012).

To enhance the growth of probiotic microorganisms, the addition of new ingredients such as tomato juice, peanut milk, soy milk, buffalo whey/soy milk and rice to dairy mixes has proved enormously effective **(Dufrene et al., 2021)**. Lactobacillus acidophilus grows much better on soya milk than on cow's milk, demonstrating the selective effectiveness of the substrates for each type of microorganism (M. J. Desmazeaud, 1993; Mital et al., 1970; Taylor et al., 2015). This explains why the supplementation of cow's milk with soy extracts has been proposed, and why many groups have investigated the production of yoghurt-type products based on soy milk **(Shelef et al., 1988)**. Bifidobacteria, on the other hand, seem to prefer cow's milk to soy milk for their growth. Lactic cultures also thrive in cabbage and carrot vegetable juices **(Tamminen et al., 2013)**.

The growth of lactic acid bacteria is influenced by a number of nutrients that vary from species to species. The growth of probiotic bacteria requires a variety of nutrients (particularly carbohydrates, proteins, vitamins and mineral elements) and consequently the improvement of organoleptic characteristics and the reduction of fermentation time (Zhang et al., 2019). Generally, the growth of *Bifidobacterium lactis* and *Lactobacillus casei* requires substrates rich in nitrogen compounds, vitamins and mineral salts. Other growth factors, such as lactulose and short-chain fructooligosaccharides known as “neosugars”, influence the development of *Bifidobacterium lactis* and Lactobacillus casei, as they inhibit the growth of Clostridium and Escherichia coli to avoid nutritional competition. The same applies to other substances such as lactosucrose, lactisol and xylooligosaccharides (Delcenserie et al., 2002).

Overall, carbohydrates, proteins, nitrogen compounds, mineral elements, vitamins and fatty acids are important nutrients to facilitate the proliferation of microorganisms in the environment (Yeboah et al., 2023).

In addition to nutritional requirements, science is proving the influence of factors such as the genetics of microorganisms to justify the rapid development of probiotic bacteria (Marco & Tachon, 2013).

Lactic acid bacteria require the presence of different carbon sources, which influence growth rate and biomass productivity. There is an effect on the expression of catabolic enzymes, where the presence of glucose strongly represses the expression of inducible enzyme synthesis. The “glucose effect” is respectively transient with permanent catabolic repression (Dalhoff, 1979). Each type of probiotic microorganism exhibits a growth preference with regard to sugar doses that are favorable to it (Farnworth, 2005). Substrates rich in starch, maltose, raffinose, fructose, sucrose and glucose are best for the growth of lactobacillus (Calderon et al., 2001). High concentrations of sugars inhibit bacterial growth, while low concentrations stimulate it, hence the need to determine a threshold concentration acting not as an antimicrobial, but rather a concentration qualifying it as a mediating agent (Mizzi et al., 2020). The best carbohydrates such as arabinose, xylose, fructose, glucose, sucrose, raffinose, galactose and lactose have been defined as carbon sources, where an example of culture on MRS medium with 10g sucrose promotes the growth of *B. lactis* (Schöpping et al., 2021). A study on the growth of probiotic bacteria in the presence of galactooligosaccharides (non-digestible oligosaccharides) shows that *B. lactis* preferentially uses tri- and tetra-saccharides, whereas Lactobacillus rhamnosus prefers sugars with a lower degree of polymerization (mono- and disaccharides), hence a substrate rich in galactooligosaccharides favors the growth of *B. lactis* (Gopal et al., 2001).

The main prebiotic agents are oligosaccharides and dietary fibres (mainly inulin) [Grizard & Barthomeuf, 1999]. Among dietary fibers with prebiotic potential, inulin and oligofructose are the most widely used in the food industry (Riazi & Ziar, 2010).

Being short-chain carbohydrates, soluble fibers stimulate the growth of bifidobacteria, are necessary for the proliferation of Lactobacillus in the intestinal tract and constitute the ideal substrate for the generation of certain metabolites essential for colon cell nutrition, such as short-chain fatty acids (Losada et al., 2002).

The most telling example is the impact of lemon and orange fibers on the integral development of lactic acid bacteria. Fiber can have a positive impact, as well as acting as an inhibitor (Esther Sendra et al., 2007).

Probiotic growth requires the presence of amino acids. Bifidobacteria require cysteine as a source of organic sulfur, as they are unable to assimilate inorganic sulfur; consequently,

methionine and cysteine can serve as the sole source of amino acids and sulfur for bifidobacterial growth (Schöpping et al., 2021).
Calcium appears to be a cation involved in the adhesive capacity of propionibacteria, and enhances the adhesion of lactobacillus bacterial cells in the gut (Zárate et al., 2002).

During the growth of *L. casei*, manganese supplementation in the form of $MnSO_4{\cdot}H_2O$ at concentrations ranging from 0.005 to 0.03 g/l reduces fermentation time while maintaining high sugar conversion and lactic acid yield (Fitzpatrick et al., 2001).

A study on milk supplementation with Magnesium salts (30mg/100g sample) showed an increase in *B. lactis* viability at the 0.05 threshold for Magnesium gluconate and Magnesium Pidolate (Szajnar et al., 2019).

Magnesium and calcium are essential minerals where Ca is the extracellular cation that is kept constant at the cellular level to aid cell regulation by maintaining physiological functions and Mg which is intracellular is involved in enzyme activation. Mg has antagonistic effects on Ca functions including Ca and K transport in cells (Sulibursk a et al., 2021).

It is therefore necessary to study the viability of probiotic microorganisms in extracts of autochthonous agricultural products, given their high mineral content.

2. Materials and methods

2.1. Collection of solid samples

Solid samples of *D. bulbifera* and *C. dysentericus* were collected from the agro-ecological zone of the KIBIRA forest in Kayanza Province.

2.2. Preparation of extracts as test samples

First, the tubers are weighed, washed and peeled. For each type of tuber, we split them in two parts. One part is used fresh, and another part undergoes cooking. Fresh and cooked substrates are all ground, manually pressed and filtered to obtain a liquid extract for use in cultivation. The cooked extract is sterilized at $131^{o}C$ for 15 minutes before being introduced into the inoculum.

Figure 1. Sorts of substrates and the way of their preparation

2.3. Determination of mineral elements

The substrate was pre-dried, weighed, calcined and mineralized in accordance with AOAC method 975.03, 21st Edition, 2019 for the determination of mineral salts in a solid sample. It was expressed in mg/kg.

2.4. Fiber determination

Soluble, Insoluble, and Total Dietary Fiber in Foods and Food Products" has been determined carried in accordance with AOAC Method 991.43, and it was expressed in percentage.

2.5. Determination of acidity

Determination of acidity is a titrimetric method involving the determination of lactic acid in a fermented product, and was carried out in accordance with AOAC 942.15, and it was expressed in Thörner degree.

2.6. Microbial cultures

L. casei JYLC-374 and *B. lactis* JYBR-190 constitute the inoculum. Each culture powder contains 10^{10} CFU/g of live cells of these bacteria. Their viability in these substrates is studied in comparison with the really conditions realized in MRS agar culture medium used as a control, with paralleled comparison with the standard which has been validated by WHO and ISO as a normal concentration of live cells in a probiotic product equal to 10^{6}UFC/ml..

2.7. Preparation of inoculum

To prepare the probiotic bacteria activation solution, 15g of peptone powder was poured into one liter of distilled water. The mixture was sterilized in an autoclave at 121°C for 15 minutes, after being stirred to obtain a homogeneous mixture. The resulting solution is used for inoculum pre-chilling and dilution preparation.

Probiotics are generally preserved in powder form. We therefore carried out pre-activation to obtain a ready-to-use liquid solution containing live microorganisms.

After preparing the necessary equipment, one gram of the powdered probiotic bacteria is taken and placed in a test tube containing 9ml of peptone water. The mixture is shaken by hand and incubated at 37°C for 24 hours.

2.8. Fermentations

1ml of inoculum was added to the petri dish, after which a liquid extract of either the substrate or the culture medium (SRM) was added as a control. The kinetic parameters at harvest were the overall content of microorganisms and the variation in acidity during product storage. Once the most competitive dilution had been identified, a practical sample was taken to collect the product's kinetic parameters. These were acidity and shelf life.

2.9. Kinetic parameters

2.9.1. Counts of probiotic bacteria

Colony counts were carried out using a SCAN® 300 automatic colony counter.

2.9.2. Statistical analyses

Determination of correlation, the degree of contribution and linear regression of kinetic parameters was performed using STATA 15 software.

3. Results and discussion

3.1 Chemical composition of substrates

The chemical composition of the substrate is the most important factor in understanding the development of probiotic bacteria.

Table 1. Minerals and fiber content of substrates.

SUBSTRATES	CHEMICAL ELEMENTS	MEAN, Mg/Kg	Std. Error of Mean	Std. Deviation	ANALYSIS OF VARIANCE	
					Prob>F	Prob>chi2
DB	Ca	1241.2a	±151.945	429.765	0.0000	0.027
CD	Ca	437.2b	±151.945	429.765		
DB	Mg	3336.475c	±243.363	688.336		
CD	Mg	2048.7d	±243.363	688.336	0.0000	0.322
DB	Na	58.4e	±9.691	83.88		
CD	Na	109.45f	±9.691	83.88	0.0000	0.714
DB	K	18299.65g	±2100.870	5942.158		
CD	K	7182.675h	±2100.870	5942.158	0.0000	0.003
DB	Fe	511.8i	±70.728	324.38		
CD	Fe	137.3j	±70.728	324.38	0.0000	0.099
DB	fibres	5.9575k	±0.756	2.138		
CD	fibres	1.53l	±0.756	2.138	0.0000	0.136

The ANOVA test justifies a significant difference in Calcium, Magnesium, Sodium, Potassium, Iron and Fiber content between DB and CD extracts, as p-value is significantly lower than 5%.

3.2. Viability of *Lactobacillus casei* JYLC-374 and *Bifidobacterium lactis* JYBR-190 in *Dioscorea bulbifera* and *Coleus dysentericus* extracts

3.2.1. Viability of Lactobacillus casei JYLC-374 and *Bifidobacterium lactis* JYBR-190 in *Dioscorea bulbifera* extract

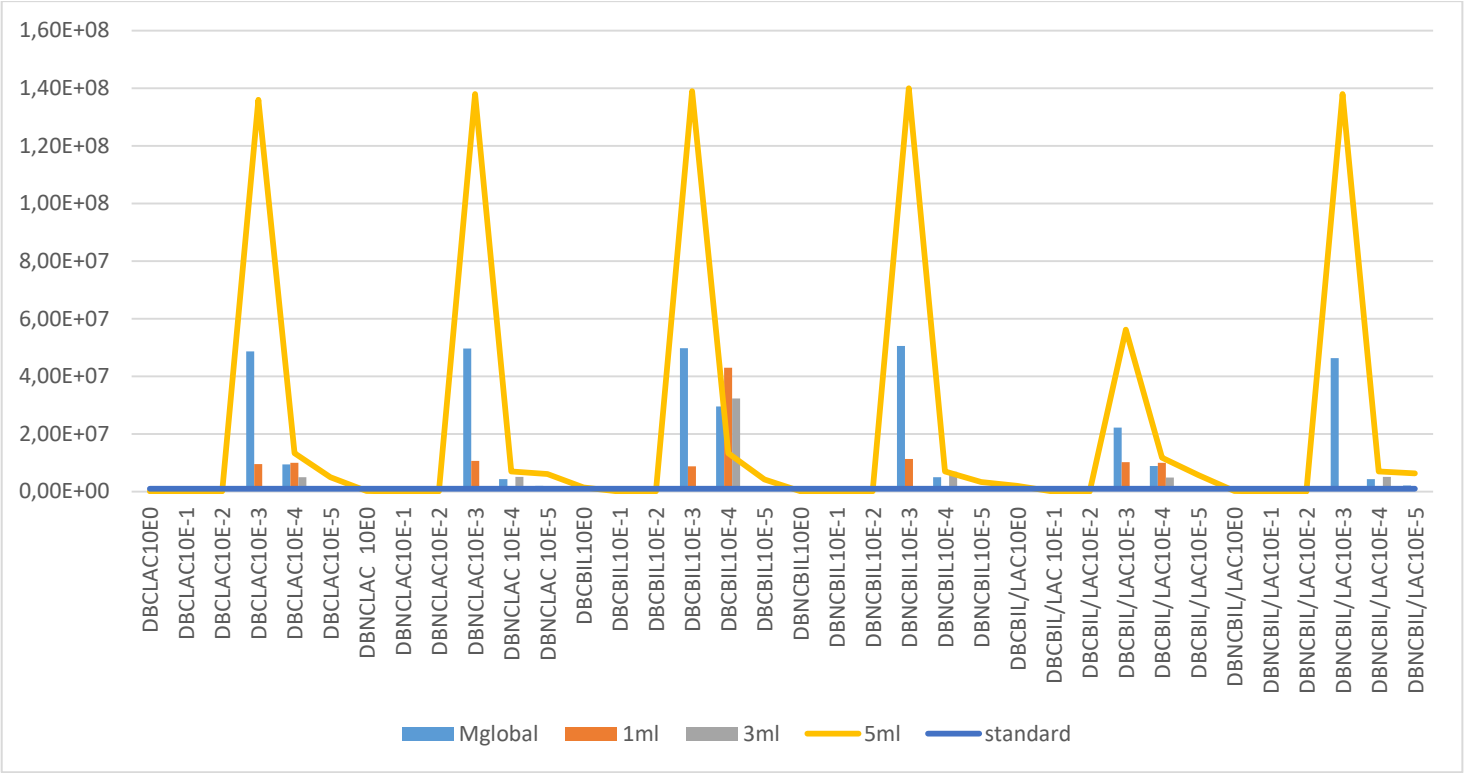


Figure 2. Test of the Viability of L. casei JYLC-374 and *B. lactis* JYBR-190 in *DB* extract

3.2.2. Viability of *Lactobacillus casei* JYLC-374 and *Bifidobacterium lactis* JYBR-190 in *Coleus dysentericus* extract

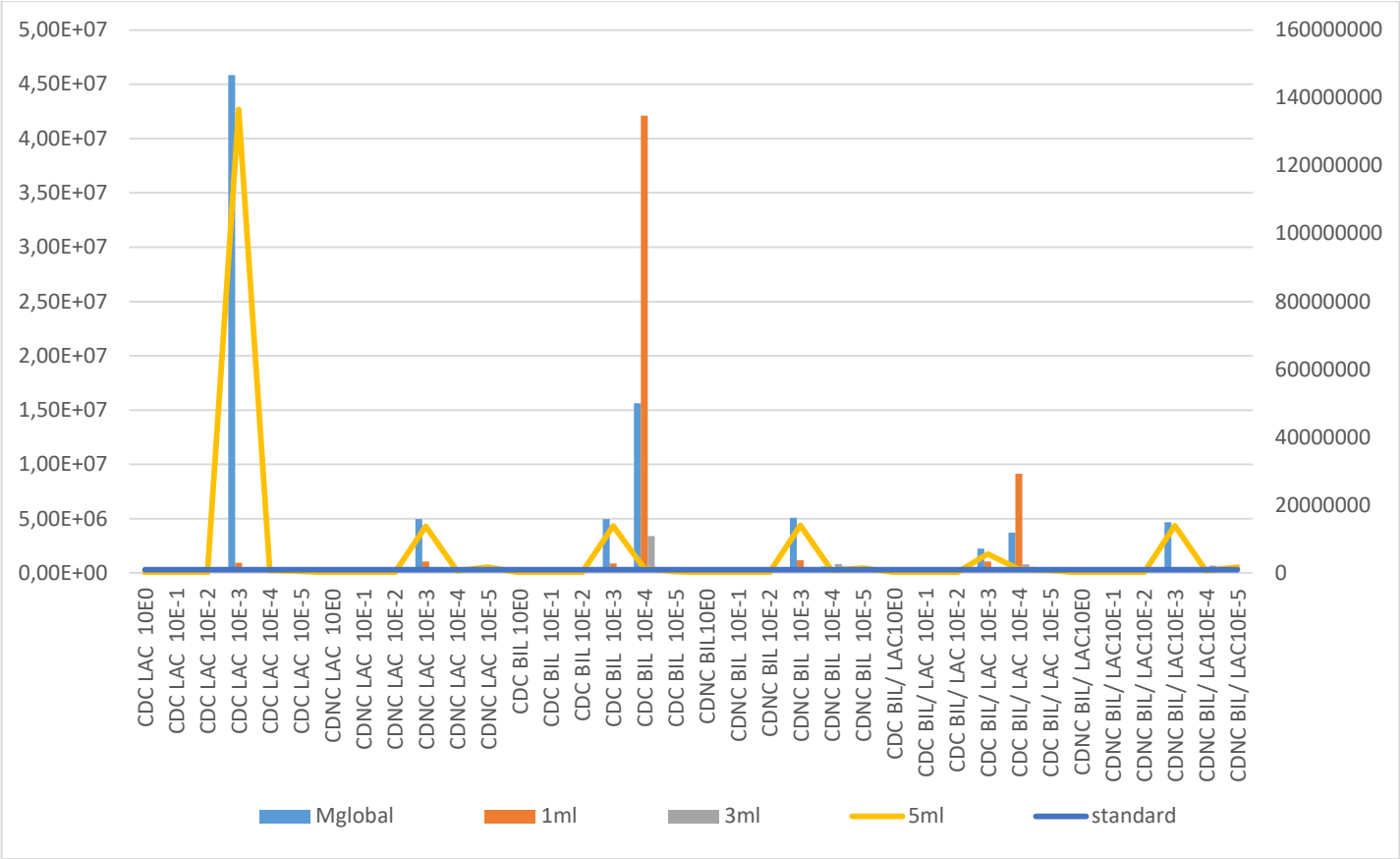


Figure 3. Test of the Viability of L. casei JYLC-374 and *B. lactis* JYBR-190 in *CD* extract

The combination of *B. lactis* and *L. casei* in different solutions of *C. dysentericus* or *D. bulbifera* extracts results in rapid growth compared to the standard threshold and this is observed at 10^{-4} dilution when using different extract concentrations. The multiple comparison test shows a non-differential trend between cooked and uncooked substrates in the growth of these bacteria, as P-value is greater than 5%. Yet the influence of viscosity on bacterial growth is enormous. The greater dilution, is more favorable on the development of microorganisms.

There is also a clear difference in the growth of bacteria in the two different solutions. *D. bulbifera* extracts showed a favorable result, with excessive viability in excess of 1000% compared with the standard for probiotic food products. The overall average (Mglobal) was always higher than 10^{6}UFC/ml, which is a standard average for probiotics.

Table 2. Impact of nutrient concentration on the growth of probiotic bacteria

Type of substrate	Fischer	P-value	R-squared	Cons.
DBCLAC10E-3	F(0,2)	0.0001	0.22	0.383
DBNCLAC10E-3	F(0,2)	0.0001	0.20	0.379
DBCBIL10E-3	F(0,2)	0.0001	0.21	0.380
DBNCBIL10E-3	F(0,2)	0.0001	0.18	0.377
DBCBIL/LAC10E-3	F(0,2)	0.0001	0.11	0.327
DBNCBIL/LAC10E-3	F(0,2)	0.0001	0.25	0.420
CDC LAC 10E-3	F(1,1)	0.0003	0.5421	0.500
CDNC LAC 10E-3	F(1,1)	0.0003	0.1932	0.710
CDC BIL 10E-3	F(1,1)	0.0087	0.2078	0.495
CDNC BIL 10E-3	F(1,1)	0.0097	0.1939	0.495
CDC BIL/ LAC 10E-3	F(1,1)	0.0058	0.1189	0.497
CDNC BIL/ LAC10E-3	F(1,1)	0.0076	0.2488	0.499

Statistical analysis using the multiple correlation test amplifies the hypothesis that mineral salts (Ca, Mg, Na, K and Fe, and fiber) have a considerable influence on the development of lactic acid bacteria, given that the p-value is close to 0. This test was carried out on all types of substrate for each concentration category and its nature (cooked or uncooked).These elements taken as dependent factors contribute from 11 to 54% to the growth of these probiotic bacteria with an average contribution of 22%.

Table 3. Impact of nutrient concentration on acid accumulation during storage

Analysis of the variance between acidity accumulation averages as a function of time storage,°Th								Degree of acidity increasing
Sample number	Sample name	DOSA/M	3 days	10 days	15 days	25 days	30 days	
0	témoins	standard	65	75	80	98	111	171%
1	CDC/LAC	3ml	57±1.5	61±2	70.3±2.6	75±2.3	81±1.5	142%
2	CDNC/LAC	3ml	69±2.3	73.3±2	79±0.5	83.3±0.8	87.3±1.4	127%
3	CDC/BIL	3ml	53±2.3	57±1.7	62.6±0.6	65.3±1.4	74.6±2.6	141%
4	CDNC/BIL	3ml	62.3±1.4	64.3±1.3	70.6±3.3	70.3±3.5	76.6±4	123%
5	CDC/BIL/LAC	3ml	62.6±1.4	64.3±1.2	71±2	73.3±2	78.6±2	125%
6	CDNC/BIL/LAC	3ml	65.3±1.4	68.6±2.4	74±2.6	78.6±0.3	82.6±1.4	126%
7	DBC/LAC	3ml	65±2.8	68.3±2.9	70±2.8	72.6±2.6	78±1	120%
8	DBNC/LAC	3ml	70±4.04	73.6±2.3	79±2	81.6±2.6	84.6±1.2	121%
9	DBC/BIL	3ml	62.3±1.4	70.3±2.6	78±4.7	65.3±1.4	73.3±1.6	118%
10	DBNC/BIL	3ml	62.6±2	65±3.2	72.3±6.2	69.6±4	75.6±5	121%
11	DBC/BIL/LAC	3ml	64±0.5	66.6±1.2	71.6±2.7	72.6±1.4	75.3±1.2	118%
12	DBNC/BIL/LAC	3ml	63.3±2.2	68.6±2.3	75±1.1	77.3±1.2	77±3.5	122%
P value			0.0013	0.0006	0.0447	0.0000	0.0000	
Average increase in acidity								125%

Acidity is measured from one day to the next, taking into account the initial acidity, which makes it possible to determine the total acidity for each day of storage.

With regard to the development of acidity during storage, the 110°Th threshold was exceeded in 30 days for the control sample. The threshold found in many cases is close to that of pasteurized milk and yoghurt, which varies from 20-110°Th in 14 days. However, a stabilization of lactic acid production was observed, which was much more pronounced in DB extracts than in CD. To clarify, the stabilizing character of the extracts studied, it is much more pronounced in DB extracts than in CD extracts. Based on the samples studied, we can confirm that fiber content is the driving force behind biochemical stabilization during cold

storage. The average increase in acidity was 125%, compared with 171% for the control sample.

Table 4. Test of comparison between the acidity levels accumulated in 3 and 30 days

```
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
      30jrs |      37    79.62162    1.254632    7.631628    77.07711    82.16613
     3jrs |      37    63.10811    .8808812    5.358191     61.3216    64.89462
---------+--------------------------------------------------------------------
    diff |      37    16.51351     1.26848    7.715863    13.94092    19.08611
------------------------------------------------------------------------------
     mean(diff) = mean(30jrs - 3jrs)                               t =  13.0183
 Ho: mean(diff) = 0                         degrees of freedom =       36

 Ha: mean(diff) < 0          Ha: mean(diff) != 0          Ha: mean(diff) > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

The averages between the acidity levels accumulated in 3 days and those accumulated in 30 days are indeed different, since P-value = 0.000 and the values in 30 days are much higher than the acidity values in 3 days.

Nevertheless, the stabilization of acidity accumulation is much more pronounced in bacteria seeded in DB extracts than in CD extracts.

Table 5. Effect of fiber cryo-protection during fridge storage of probiotic products

	BIL/LAC averages as a function of time and substrate concentration					
Sample name	3 days	10 days	15 days	25 days	30 days	Degree of survival
Control	8.94E+10	6.26E+06	1.10E+05	4.40E+03	6.00E+01	0.0000%
CDC LAC	7.73E+06	1.56E+06	2.42E+05	3.11E+04	1.92E+04	0.2485%
CDNC LAC	997866.7	5.90E+04	2.18E+04	1.44E+03	3.43E+02	0.0344%
CDC BIL	3.50E+06	2.50E+05	5.73E+04	1.75E+04	3.51E+03	0.1002%
CDNC BIL	8.90E+05	2.10E+05	8.67E+04	2.97E+04	1.13E+04	1.2734%
CDC BIL/ LAC	1.05E+06	2.50E+05	6.10E+04	3.63E+03	2.07E+03	0.1977%
CDNC BIL/ LAC	9.50E+05	1.80E+05	7.17E+04	7.07E+03	1.07E+03	0.1126%
DBCLAC	1.00E+07	2.84E+06	1.40E+06	8.10E+05	3.33E+05	3.3333%
DBNC LAC	9.38E+06	2.93E+06	1.42E+06	3.93E+05	1.67E+05	1.7766%
DBC BIL	1.36E+07	3.60E+06	1.67E+06	7.57E+05	1.33E+05	0.9804%
DBNC BIL	9.46E+06	2.57E+06	8.40E+05	8.17E+05	3.89E+05	4.1070%
DBC BIL/LAC	5.61E+06	2.00E+06	4.77E+05	3.28E+05	1.51E+05	2.6840%
DBNC BIL/LAC	3.87E+07	9.89E+06	7.17E+06	5.80E+06	3.66E+06	9.4651%

Averages of microorganisms during refrigerated storage show that both DB and CD extracts have a protective effect on probiotic bacteria. The control sample showed a small lever of survival for either type of bacteria after 30 days of storage. However, there was a protective effect for BIL and LAC seeded in DB and CD extracts, as the average survival rate reached just over 0.03% at 9% during 30 day's storage at 6°C. This protective effect is the result of fiber concentration, as fibers are more concentrated in DB than in CD. The greatest protective effect was observed in DB extracts compared with CD extracts.

Table 6. Test of comparison between the microorganism's development in 10 and 30 days

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
30jrs	37	50361.11	27059.04	164593.7	-4517.171	105239.4
10jrs	37	1818527	453732.1	2759944	898315.7	2738738
diff	37	-1768166	440938.9	2682127	-2662431	-873900.4

mean(diff) = mean(H - E) t = -4.0100
Ho: mean(diff) = 0 degrees of freedom = 36

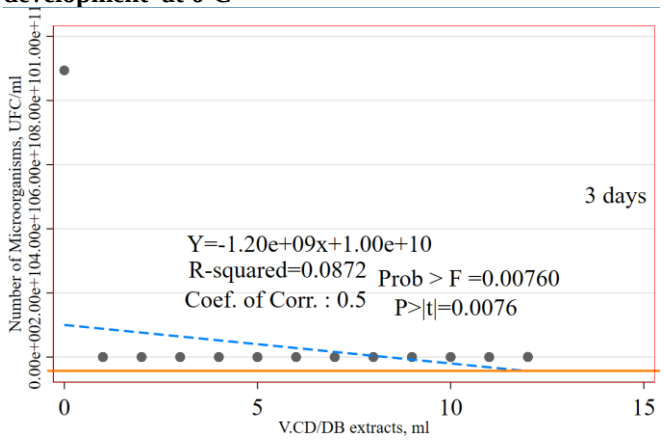
Ha: mean(diff) < 0 Ha: mean(diff) != 0 Ha: mean(diff) > 0
Pr(T < t) = 0.0001 Pr(|T| > |t|) = 0.0003 Pr(T > t) = 0.9999

When the product is stored at 6°C, the averages of microorganism's development as a function of time differ widely, P-value being equal to 0.0003. However, the average number of microorganisms surviving thirty days is much lower than the average number surviving 10 days in the refrigerator.

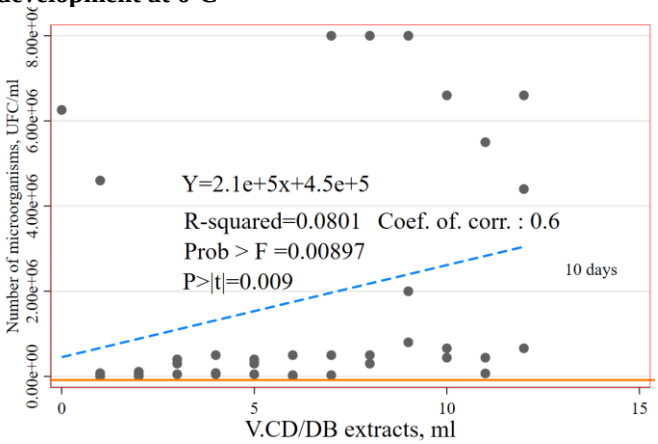
Survival was more pronounced in microorganisms grown in DB extracts than in CD extracts, in accordance with the survival percentage data given in the above table.

3.3. Cryo-protective effect of CD and DB extracts on the stabilization of microorganism's development during cold storage

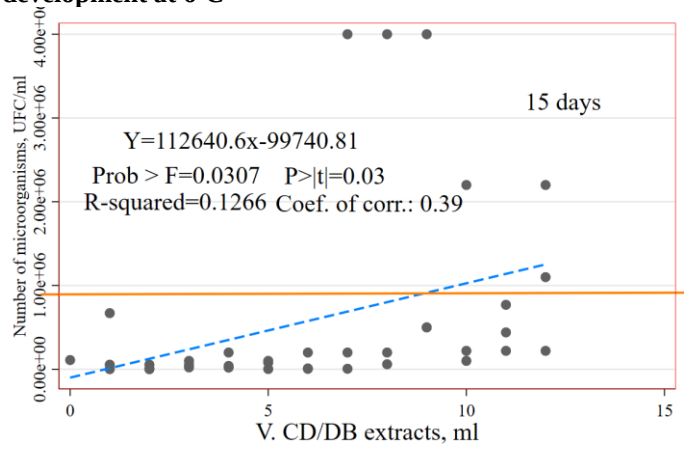
Effect of substrate extracts on three days microorganism's development at 6°C



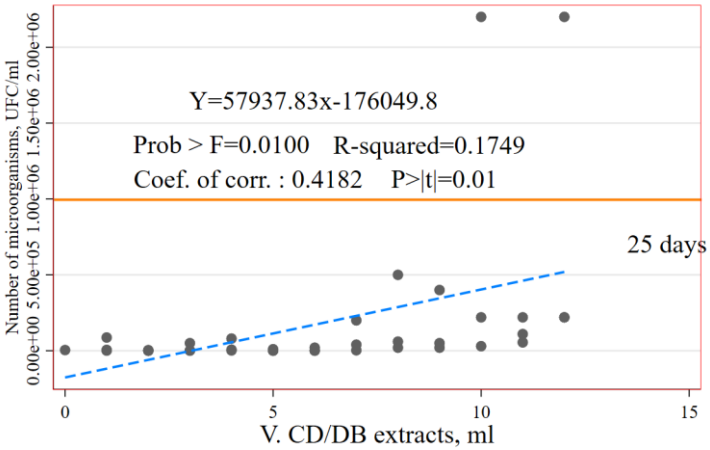
Effect of substrate extracts on ten days microorganism's development at 6°C



Effect of substrate extracts on fifteen days microorganism's development at 6°C



Effect of substrate extracts on twenty five days microorganism's development at 6°C



Effect of substrate extracts on thirty days microorganism's development at 6°C

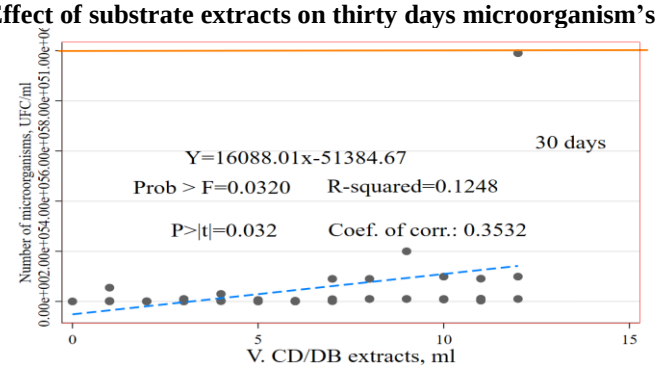


Figure 4. Comparison of the cryo-protective effect of CD and DB extracts at different days of storage

The microorganisms developed efficiently during the three-day incubation period, exceeding the threshold of 10^6UFC/ml recommended by WHO structures for foods with probiotic connotations. The influence of extracts on the development of microorganisms is real, as the p-value is less than 5%. The contribution of CD or DB extracts averaged 12% in the first three days of microorganism's development under thermal incubation conditions, i.e. at 37°C. The

control sample exceeded the 10^6UFC/ml threshold twice, while the substrates used for the study hovered around 10^7UFC/ml.

After three day's incubation, the products were stored in the fridge at 6°C.

Analysis of the development of microorganisms during cold storage revealed that the level of microorganisms exceeded the previous threshold, suggesting that the substrates were cryo-protective towards microorganisms. On the day of preservation, the microorganism's content remained above the WHO recommended threshold which is 10^6UFC/ml.

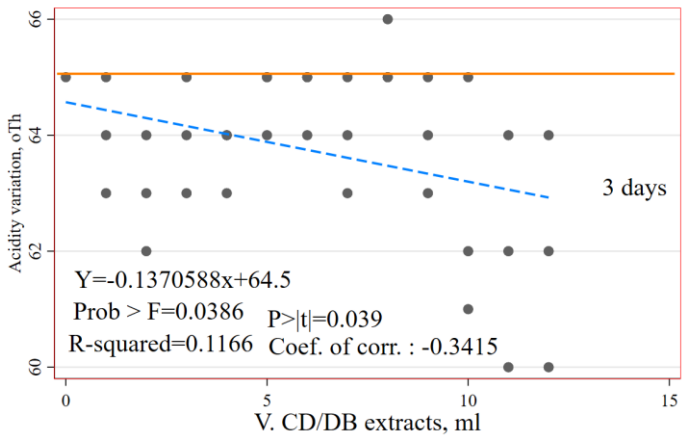
At day 15, we notice a depletion of nutrients. In the control sample, the microorganisms are considerably depleted and fall far below the WHO recommended threshold. However, in the samples studied, the cryo-protective effect remains, as the microorganism's content remained above the threshold in some samples.

After 25 day's cold storage, we can still see the cryo-protective effect, as some samples were able to maintain a number of microorganisms above the WHO recommended threshold, while the control sample already had a content close to 0 UFC/ml.

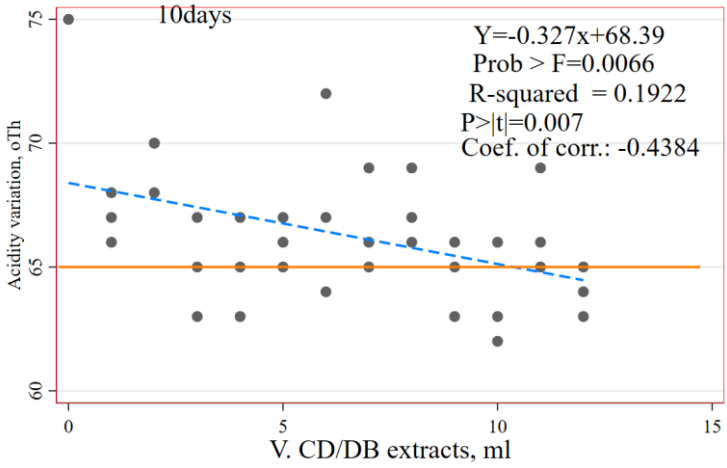
At the 30th day of refrigerated storage, none of the samples studied met the required conditions, as all the samples analyzed contained microorganisms below the 10^6UFC/ml threshold. But comparing the results, the development in DB and CD extracts stay more effective than in the control sample. And the more related effect was noticed in DB extract than in CD extract. So that the cryo-protective effect was more effective in DB extracts than in CD extracts.

3.4. Stabilizing effect of CD and DB extracts on acidity production during product cold storage

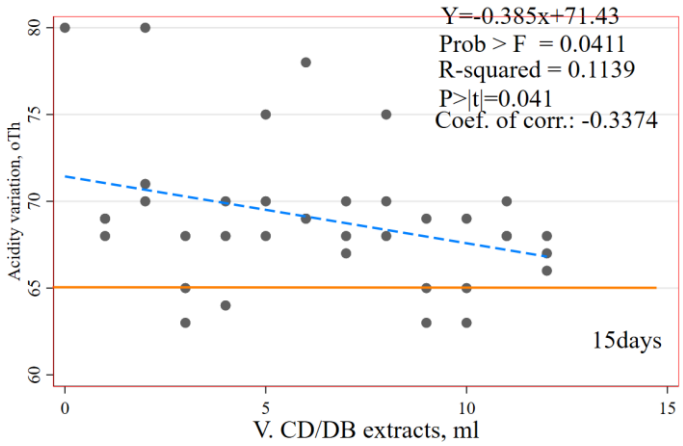
Effect of CD or DB extracts on 3 days acidity variation at 6^0C



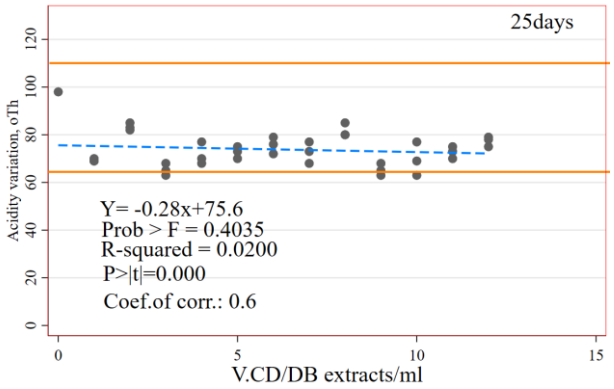
Effect of CD or DB extracts on 10 days acidity variation at 6^0C



Effect of CD or DB extracts on 15 days acidity variation at 6^0C



Effect of CD or DB extracts on 25days acidity variation at 6^0C



Effect of CD or DB extracts on 30 days acidity variation at 6^0C

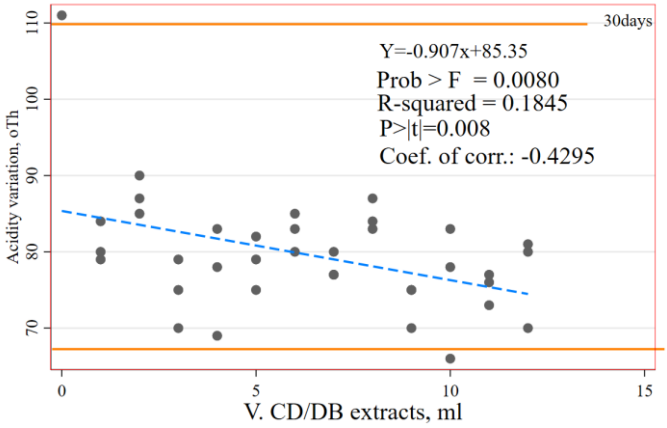


Figure 5. Comparison of the stabilizing effect of CD and DB extracts at different days of storage

By the 3rd day of fermentation, all samples had reached the threshold acidity of 65°Th. After 10 days of storage, acidity increased with an exponential relationship that was much more pronounced in the control sample. After 15 days of product storage, we note an accelerated development of acidity in all samples, while some samples still keep acidity close to the threshold. In this context, it is acceptable that the stabilizing character is also demonstrated in the management of biochemical reactions of the product during storage. As extract content increases, so does stabilizing capacity.

After 25 days of cold storage, almost all products have already exceeded the minimum threshold, but all samples remain within the tolerable limits for the maximum threshold of 110°Th.

At 30 days, only the control sample had exceeded the tolerable acidity limits (110°Th). All other samples experienced an exaggerated slowdown in acidity accumulation. Nevertheless,

as extract content increases, stabilizing capacity increases, with a linear descending equation to justify its progressive tendency to 0 as a function of time.

4. Conclusion

The present study has showed the possibility of probiotic microorganisms like *Bifidobacterium lactis* JYBR-*190* and *Lactobacillus casei* to be developed in some local substrates extracts. Considering the two substrates studied, the DB extracts has shown a good response of microorganism's development more than CD extracts. The extracts of those two substrates succeed to develop those probiotics microorganisms because of their content in minerals elements and fibers. Statistics interpretation of results allowed to summerize a stabilizing effect according to the acidity development while product storage after incubation. It has also shown the cryo-protecting effect towards probiotics microorganisms studied in this work. We comes out our conclusion that as fibers are a lot as cryo-protecting and stabilizing effect are higher, so that effect was more effective in DB extracts than in CD extracts. Then local traditional subtracts like DB and CD can be used to promote the probiotics products as main constituents or as additives elements.

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