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Influence of Different Commercial Starter Cultures On Quality of Yogurt

A. DABIJA[1], G.G. CODINĂ[1], A. M. GÂTLAN[1]
[1]Faculty Of Food Engineering, Ștefan Cel Mare University Of Suceava,
13th University Street, 720229, Suceava, Romania
Corresponding Author: A. Dabija

Abstract: *The Starter Culture Is The Most Important Factor For Determination Of The Overall Quality Of Yogurt, Defining Its Qualitative And Nutritional Characteristics And Also Determining The Type Of Fermentation Process And The Final Fermentation Metabolites. Regularly, For The Yogurt Production, There Are Used Symbiotic Cultures Consisting Mainly Of Lactobacillus Bulgaricus And Streptococcus Thermophilus Bacteria, In Specific Growth Conditions. That Is Used Raw Milk From A Local Dairy Producer, For Obtaining Samples Of Yogurt. In Technological Process Were Used Three Different Commercial Starter Cultures That Represent Object Of Present Study. Yogurt Samples Were Refrigerated And Then Analysed After 24 Hours Of Storage, From The Point Of View Physicochemical, Sensorial And Rheological Properties. The Results Were Performed Statistically Using One-Way Analysis Of Variance (ANOVA). Final Conclusions Had Permitted Selection Process Of The Best Starter Culture For Industrial Process Of Yogurt.*

Keywords - *Biotechnological Properties, Functional Product, Rheological Properties, Syneresis*

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I. Introduction

For A Long Time, Fermented Dairy Products, Were Primarily Designed To Stabilize The Milk From The Point Of View Biochemical And Microbiological, And Also To Diversify The Range Of Dairy Products, Which Demonstrate Nutritional Functional Foods [1-4].

FAO/WHO Codex Alimentarius Commission Definition Of Yogurt Is: "A Coagulated Milk Product Obtained By Lactic Acid Fermentation Through The Action Of *Lactobacillus Bulgaricus* And *Streptococcus Thermophilus* From Milk (Pasteurized Or Concentrated Milk) With Or Without Additions". The Microorganisms In The Final Product Must Be Viable And Abundant [5]. Symbiotic Interaction Called Proto-Cooperation Is One Of The Key Factors Which Determine The Fermentation Process And The Final Quality Of Yogurt [6-8]. The Reactions Of Bacteria Activity Lead To The Production Of Various Metabolites Resulting In Decrease Of Ph And The Formation Of Texture, Taste And Aroma.

Starter Cultures Have A Prime Role In Defining Qualitative And Nutritional Characteristics Of Fermented Dairy Products, Determining The Type Of Fermentation Process And The Final Fermentation Products [9, 10]. The Two Species Bacteria Ferment Almost All The Lactose To Lactic Acid And Flavour The Yogurt With Diacetyl By *Streptococcus Thermophilus* And Acetaldehyde By *Lactobacillus Bulgaricus*. Contributions Of The Two Bacteria To The Integrated Metabolism Have Been Studied And Several Components Identified [11].

Streptococcus Thermophilus Produces Pyruvic Acid, Formic Acid, Folic Acid Which Reduces The Ph Of Milk To An Optimum Level For Growth Of *Lactobacillus Bulgaricus*. In The Same Time, Growth Of *Lactobacillus Bulgaricus* Supplies Peptides, Free Amino Acids And Putrescine That Stimulate Growth Of *Streptococcus Thermophilus* [12-14].

Several Studies Reported That The Exopolysaccharides Production Is Ranging From 55 To 150 Mg/L In Fermented Milk. The Parameters Of Fermentation Play An Important Role In Metabolism Of *Lactobacillus* During The Growth, And It Affects The Uptake Of Nutrients, Protein Synthesis, Glycolysis And Nucleic Acid Synthesis [15- 19]. Generally, Lactic Acid Bacteria Are Known To Possess Antimicrobial Activities, To The Decrease Of Ph Due To Fermentative Process And To The Production Of Inhibitory Metabolites [20-23].

Lately, Yoghurt Has Been Used As A Vehicle For Probiotic Bacteria. Except Of These Two Bacteria Used To Obtain Health Benefits From Probiotic Products, Recent Trend Is Using 5 To 6 Different Strains Of Probiotic Organisms [24]. Was Demonstrated Antimutagenic, Anticarcinogenic And Anti-Diarrhoeal Properties, Immune System Stimulation, Reduction In Blood Pressure, Reduction In Serum Cholesterol Concentration, Increased Resistance To Infectious Diseases, Growth Stimulation, Improvement In

Gastroenteritis/Inflammatory Bowel Disease And Suppression Of *Helicobacter Pylori* Infection, Maintenance Of Balanced Flora And Improvement In Lactose Metabolism In Yogurt, Given By Those Bacteria [25, 26].

Maintaining A High Level Of Viable Probiotic Cell Count In Yogurt, Along The Shelf Life Is Not A Simple Task**.** The Viability Of Lactic Acid Bacteria In Yogurts Are Mainly Influenced By Several Factors Such As: Strain Variation, Acid Accumulation, Interaction With Starter Cultures, Level Of Dissolved Oxygen And Storage Condition [25, 27].

Using A Mixed Culture Mode, In Order To Obtain The Starter Culture Of Lactic Bacteria Is Less Costly, And Also Facilitates The Achieving Of Favourable Ratio Of These Bacteria. Several Factors For Bacteria Metabolism And Growth Are: Temperature And Ph During Fermentation Process, Product Formation Of Lactic Acid Bacteria And Medium Composition [28-30].

The Main Purpose Of This Paper Was To Perform A Comparative Study Of Some Qualitative Characteristics Of Yogurts Obtained By Using Different Commercial Bacteria Starter Cultures.

II. Materials And Methods

2.1. Materials

Obtaining The Yogurt Sample Respects The Technological Process Of The Local Dairy Producer"S.C. TUDIA S.R.L. Suceava". It Was Used Raw Cow's Milk With Following Parameters: Fat Content Of 3.5%, 3% Protein And 4.5% Carbohydrates. The Starter Cultures Were Provided By The Dairy Producer, In Order To Establish The Optimum Formula For The Best Quality Characteristics For Yogurt. All The Three Starter Cultures Contain *Lactobacillus Bulgaricus* And *Streptococcus Thermophilus* In Different Proportion, Present Slightly Different Biochemical Properties That Are Confidential, For An Objective Final Selection Process. The Starter Culture Ratio Used In Technological Process In The Laboratory Scale, Respects Also The Receipt Of Producer That Is 1 % Level (W/V).

2.2. Samples' Preparation

Yogurt Samples Were Developed And Produced Inside Food Technologies Laboratory That Belongs With Food Engineering Faculty ("Ștefan Cel Mare" University Of Suceava, Romania). The Preparation Method For Yogurt Samples Was Performed As Following: Raw Milk Was First Pasteurized At 90°C For 15 Minutes And Cooled To 42°C. The Heat-Treated Milk Was Divided In 3(Three) Lots, Each Of Them Being Inoculated With A Constant Percent Of Starter Culture Of Specific Bacteria, Of 1%(W/V). Fermentation Process Was Carried Out At 42±0.2ºc, With Monitoring Ph, Every 30 Minutes. After The Ph Value Reached 4.6±0.2 (The Period Of Time Is In The Range Of 2.5-3 Hours), Yogurt Was Refrigerated At 4±0.5°C And Stored At The Same Temperature. The Yogurt Samples Were Identified By Letters "A"; "B"; "C", Depending Of The Starter Culture Used. All Samples Were Done In Triplicate.

2.3. Methods

The Analyses Performed Were: Physicochemical (Ph; Total Acidity; Syneresis; Whey Holding Capacity); Sensorial And Rheological Properties. We Are Used Equipments That Respect The Requirements In The Method Standards. The Monitoring Of Ph Was Conducted Using A Ph Portable F2 Standard Mettler Toledo Device (STAS 8201-82). The Titratable Acidity Determination Was Made By Respecting The Following Classical Method: A Blend Of 10 Ml Of Yogurt Samples, 20 Ml Distilled Water And 3 Drops Of Phenolphthalein Were Titrated With Naoh 0.1 N Until The Stabilization Of A Pale Pink Colour. The Results Were Expressed As Thörner Degree.

For The Syneresis Determination There Were Used 10 Ml Of Yogurt Which Were Weighed. Yogurt Samples Were Placed In Tubes Of Spin MPW 223E Centrifuge And Centrifuged At 639 × G For 10 Min, At 4±1°C. The Clear Supernatant Was Poured Off, Weighed And Expressed As Percent Weight Relative To Original Weight Of Yogurt To The Following Equation [31]:

Syneresis (%) = [(Weight Of Supernatant / Weight Of Yogurt Sample)] X 100 (1)

The Whey Holding Capacity Of The Yogurt Samples Was Determinate Utilizing A Method Developed By Trejo *Et Al.* (2014) [32]. The Yogurt Samples Were Weighed And They Were Centrifuged Of 5 G At 4500 × G For 15 Min At 4±1°C. The Whey Holding Capacity (WHC) Was Calculated Utilizing The Following Equation: Water Holding Capacity Of Yogurt Samples Was Determined By The Centrifugation:

WHC (%) = [(Initial Weight Of The Sample – Weight Of The Removed Whey)/ Initial Weight Of The Sample] X 100 (2)

All Analysis Was Performed In Triplicate And The Mean Value Was Used For The Graphical Representation Of Data.

For Sensorial Analysis, Yogurt Samples Were Evaluated By A Consumer Panel Of 10 Students And 5 Academic Staff Of The "Ștefan Cel Mare" University Of Suceava (Among Them, 8 Females And 7 Males, With Ages Between 21 And 55 Years), That Are Trained In Conformity With SR 6345-1995, The Quality

Assessment System Being The Scoring Method And The Scale Is From 0 To 20 Points, According To Standard. The Procedure For Sensorial Analyse Was Done Following This: A Volume Of 30 Ml Yogurt Sample Was Served At 10 ± 1°C In Glass Beakers, Each Sample Being Labelled With A Code Number. According To This Method, To Each Sample Was Assigned Points From 0 To 5 And A Coefficient Of Importance (Weighting Factor) As Follows: For Appearance, Colour, Consistency And Smell $F_w = 0.5$, And For Taste $F_w = 2$. The Non-Weighted Average Score ($S_{a/N-W}$) Was Calculated For Each Characteristic, By Adding The Points Given By The Tasters To An Arithmetic Mean. The Weighted Average Score Was Calculated By Weight Factors, Multiplying Each Non-Weighted Average Score Of Each Sensory Feature By The Corresponding Weight Factor: $S_{a/W} = S_{a/N-W} \times F_w$. The Total Weighted Average Score Was Calculated By The Sum Of All Weighted Average Scores Corresponding To The Sensory Attributes Of An Analyzed Sample.

On The Basis Of The Overall Average Score, The Assessment Of The Qualitative Level Of Each Sample, From One Sensory Property, Was Compared To The Standard, As Described In SR 3665-1999.

Rheological Properties Of Yogurt Samples - Thixotropy, The Hysteresis, The Viscosity - Were Studied By Means Of A Modular Advanced Rheometer System (Thermo Haake Mars), Equipped With Geometry Plate/Plate, With 40mm Diameter And A Peltier Temperature Module. All Analyses Were Performed In Duplicate, At 8°C.

There Were Conducted Thixotropy Tests, By Monitoring The Variation Of Flow And Viscosity As A Function Of Shear Rate, Which Was Set From 0.02 S^{-1} To 100 S^{-1} (For Obtaining The Rising Curves) And From 100 S^{-1} To 0.02 S^{-1} (For Obtaining The Descending Curves). The Total Analysis Took Approximately 20 Minutes, With The Attainments Of 80 Graphical Points At 1mm Gap.

The Hysteresis Was Calculated As The Area Between The Curves And The Viscosit Was Determined As A Function Of Time For A Period Of 10 Minutes, At A Constant Shear Rate Of 100 S^{-1}, Collecting 40 Graphical Points.

Before Performing Any Rheological Analysis, The Samples Were Stored At 8°C For 10 Minutes. For Obtaining The Curves And Adjusting The Models, It Was Used The Software Haake Rheowin Data Manager. The Results Were Fitted To The Models Bingham (3), (4); Ostwald Weale (5), (6) And Herschel-Bulkley (7), (8), Following The Equations:

Bingham: $\eta = \eta_p + \tau_0/\gamma$ (3) $\tau = \tau_0 + \eta_p\gamma$ (4)

Ostwald Weale: $\eta = K\gamma^{n-1}$ (5) $\tau = K\gamma^n$ (6)

Herschel-Bulkley: $\eta = \tau_0/\gamma + K\gamma^{n-1}$ (7) $\tau = \tau_0 + K\gamma^n$ (8)

Where: H = Viscosity (*Pa.S*), η_p = Plastic Viscosity, τ = Shear Tension (*Pa*), τ_0 = Yield Strength, γ = Shear Rate (S^{-1}), K = Consistency Index ($Pa.S^n$), N = Behaviour Index (*Dimensionless*) [33,34].

Statistical Analysis

Results Are Expressed As Mean ± Standard Deviation (SD). One-Way Analysis Of Variance (ANOVA) Followed By Post Hoc LSD Test Was Carried Out To Examine The Difference Among The Means Using IBM SPSS Statistics (Version 16.0). Significance Was Defined At Level Of $P < 0.05$.

III. Results And Discussions

Yogurt Samples Were Analysed After 24 Hours Of Storage Under Refrigeration Conditions. It Was Determined The Influence Of Different Starter Culture On Physicochemical, Sensorial And Rheological Properties Of Yogurt.

3.1. Physicochemical Characteristics Of Yogurt

The Results For Physicochemical Properties Are Shown In Table 1.

Table 1. Physicochemical Properties Of The Yogurt Samples

Physicochemical Characteristics	Sample With Starter Culture:		
	A	B	C
	Mean Value ± Standard Deviation (SD)		
Ph	4.43 ± 0.02	4.46 ± 0.01	4.74 ± 0.02
Acidity, ^{O}t	127 ± 1	119 ± 1	115 ± 2
Acidity, G Lactic Acid/100 Ml	1.14 ± 0.02	1.05 ± 0.05	1.05 ± 0.10
Sineresys, %	48.20 ± 0.30	46.09 ± 0.07	44.08 ± 0.04
Whey Holding Capacity, %	51.71 ± 0.08	53.90 ± 0.60	55.91 ± 0.11

The Highest Ph Level Was Observed For The Yogurt Samples C And A Had The Lowest Ph Level. In Order To Control The Acidity Correspondent To Lactic Acid Formed In The Product, It Is Proper To Determine The Titratable Acidity, Since This Value Gives A Rapid Indication Of Changes In The Activity Of The Starter. The Acidity Developed In Yogurt Was The Result Of Bacterial Activity And Also Relevant For The Level Of Its Activity [35]. The Range Of Acidity For The Studied Samples Was Between 115 And 127 ^{0}T, Correspondent To The Indications For Ph Determinations, The Yogurt Sample B Having Acidity With 6.3% Less Than Yogurt Sample A, And Respectively, The Yogurt Sample C Acidity Was 9.44% Lower Than The Acidity Of Yogurt Sample A. Considering The Same Conditions Of Fermenting Process And The Same Period Of Time For Samples Storage, The Different Results For Ph Values, Respectively For Titratable Acidity Of The Yogurt Samples, Tells About Slight Differences On The Bacterial Activity Of The Starter Cultures Used.

Syneresis Is A Natural Phenomenon That Means Separation Of Two Phases In A Suspension Or Mixture. It Naturally Occurs In Dairy Products, Such Yogurt, And Represents An Important Attribute In Determining The Quality Of Yogurt And Other Dairy Products [36]. Using Of Different Starter Cultures Could Influence The Yogurt Gel Structure. The Results For This Parameter, Syneresis, Could Be Shown In Table 1: The Most Pronounced Gel Firmness And Hence, The Minimal Syneresis Were Observed For Yogurt Sample C, Followed By Yogurt Sample B.

In Good Correlation With Syneresis, The Whey-Holding Capacity Shows Following Values: 51.71% - Yogurt Sample A, 53.90% - Yogurt Sample B And 55.91% - Yogurt Sample C, In Conformity With Table1.

In Order To Verify If There Are Differences Between The Three Different Starter Cultures Regarding The Physicochemical Characteristics Of The Yogurt Samples, The One-Way ANOVA Method Was Applied. The Results Show That There Are Significant Differences ($P < 0.0001$) Between The Ph Of The Yogurt Sample A And Yogurt Sample C ($P >0.05$), But There Were No Significant Differences ($P > 0.05$) Between The Ph Of The Yogurt Sample A And Yogurt Sample B. Concerning Acidity (°T), Significant Differences ($P < 0.0001$) Were Obtained Between The Acidity Of Samples A And B, Respectively Sample C. Also, The Acidity Of The Sample B Differs Significantly From The Acidity Of The Sample C ($P < 0.05$). There Are No Significant Differences ($P > 0.05$) Between The Samples A And B, Respectively Sample C, But Neither Between Samples B And C In Terms Of Acidity. The Results Obtained For Syneresis Reveal Statistically Differences ($P < 0.0001$) Between Yogurt Samples A And B, Respectively Yogurt Sample C, But Also Between Yogurt Samples B And C. Whey Holding Capacity Showed Significant Differences Between Yogurt Samples A And B, Respectively Yogurt Sample C, But Also Between Yogurt Samples B And C.

Could Be Concluded That, After Physicochemical Analyses, Sample ”C” Had Shown The Best Results, Also For Syneresis And For Whey Holding Capacity, With The Lowest Value For Titratable Acidity. This Could Be Strongly Related With A Better Enzymatic Activity Of Starter Culture, Considering The Same Parameters For The Whole Process, Also For Production And For Analyzing The Yogurt Samples.

3.2. Sensorial Characteristics Of Yogurt

The Sensorial Analysis Of Yogurt Samples Really Completes Their Qualitative Evaluation. The Tasters' Panel Was Formed From 15 Persons Who Were Previously Trained With The Conditions And Methodology Of Standards. Fig. 1 Presents The Final Results Of The Sensorial Evaluation Of The Yogurt Samples. Yogurt Samples With The Highest Acceptance Scores, 19.25 Was For Sample C, Followed By The Sample B (18.24) And **The** Sample A (15.68).

As It Could Be Seen, Sensorial Analyses Demonstrates A Highest Level Of These Parameters For Yogurt Sample C, Too. A High Quality Yogurt Can't Have Good Physicochemical Properties And Poor Sensorial Characteristics And Vice-Versa.

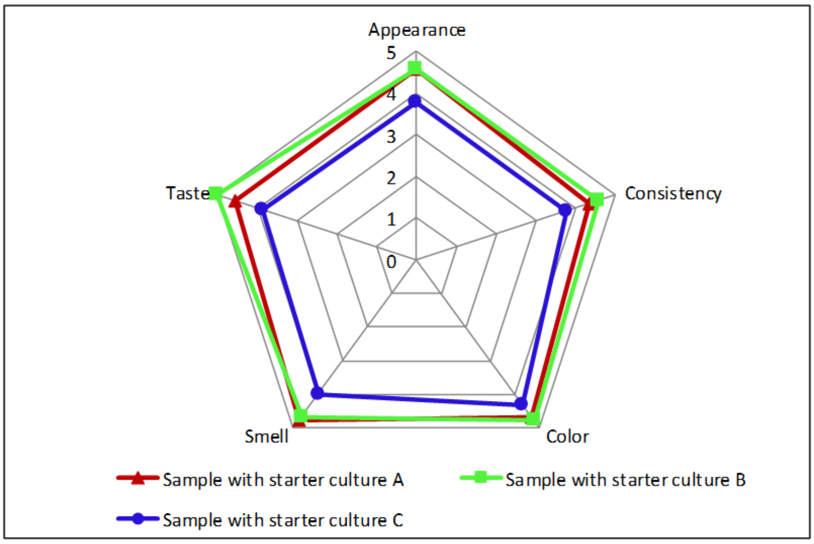

Figure 1. The Results Of The Sensory Analysis Of The Yogurt Samples (Non-Weighted Average Score)

3.3. Rheological Properties Of Yogurt

All Analyzed Samples Showed Non-Newtonian, Pseudo-Plastic Behaviour With Increasing Shear Rate Applied, Independent Of The Starter Culture Used. According To Mathias *Et Al.* (2011), This Phenomenon Could Be Caused By Low Interaction Energy Between The Yogurt Molecules, As Well As The Physical Destruction Of The Weak Bonds Between Them [33].

The Flow And Viscosity Curves For The Yogurt Samples Are Represented In Figure 2 And 3.

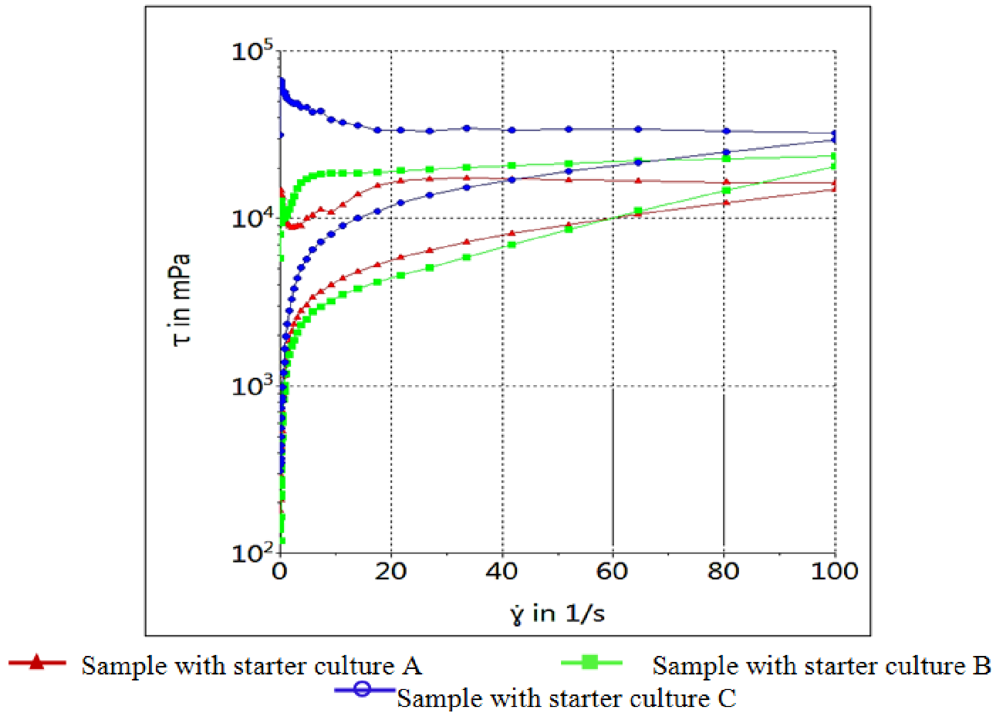

Figure 2. Flow Curves For The Yogurt Samples

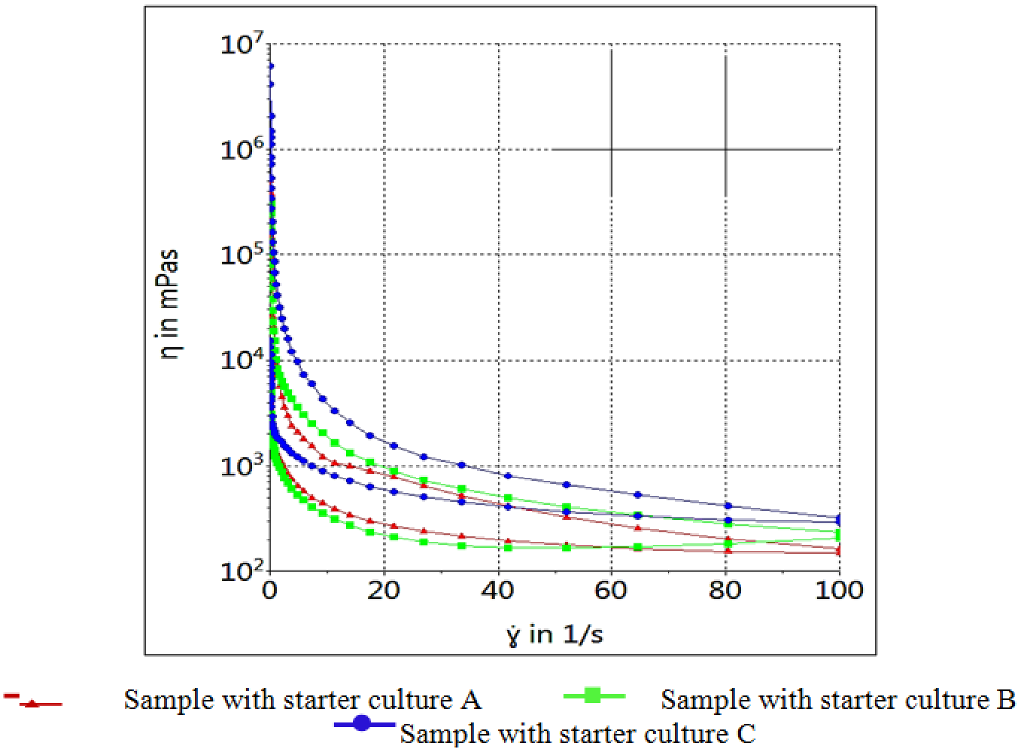

Figure 3. Viscosity Curves For The Yogurt Samples

The Yogurt Sample C With The Highest Level Of Viscosity, Over The Entire Interval Of Applied Shear Rates (0.02 S^{-1} To 100 S^{-1}), Had Confirmed The Lowest Syneresis Value Of The Sample.

All Samples Showed Thixotropic Characteristics, As There Are Differences Between The Rising And The Descending Curves Of Both Tension And Viscosity Parameters. This Phenomenon Can Be Quantified As The Area Between The Flow Curves (Hysteresis), Values That Are Given In Table 2. Also, It Could Be Observed The Flow And Viscosity Values Of Samples At 51.94 S^{-1} Shear Rate (Middle Of Examined Range Of Shear Rate), On Both Rising (↑) And Descending (↓) Curves.

Table 2. Values Of Examined Thixotropic Parameters

Sample	Hysteresis (Pa/S)	H (Mpa) At Γ = 51.94 S^{-1}		T (Mpa) At Γ = 51.94 S^{-1}	
		↑Γ	↓Γ	↑Γ	↓Γ
A	ΔA = 700.3 A↑ = 1589 A↓ = 889.1	326.8	176.8	16970	9183
B	ΔA = 1135 A↑ = 2072 A↓ = 936.8	409.5	165.9	21270	8616
C	ΔA = 1684 A↑ = 3494 A↓ = 1810	656.1	367.1	34070	19070

The Largest Hysteresis Area Was Observed For The Sample C (1684 Pa/S), Sample That Shows The Highest Tension And Viscosity Values When 51.94 S^{-1} Shear Rate Is Applied. The Sample B Had A Hysteresis Area With 32.6% Smaller Than The First Mentioned, And Finally The Sample A Had A 700.3 Pa/S Area, Which Is With 58.41% Smaller Than The Sample C. Table 3 Show The Values Of Regression Coefficient *R* For Models Adjusted For The Upward Viscosity Curves Of Yogurt Samples.

Table 3. Values Of Regression Coefficient *R* For The Models Adjusted To The Viscosity Upward Curves

Model	Sample		
	A	B	C
Bingham	0.9908	0.9511	0.9533
Ostwald Weale	0.9288	0.9787	0.9483
Herschel-Bulkley	0.9982	0.9871	0.9925

In All Cases, The Best Results Were For The Herschel-Bulkley Model (R>0.99). The Exception Was The Sample B, Its *R* Value For All Tested Models Being Below 0.99.

IV. Conclusions

By Using Three Different Commercial Starter Cultures, Assuring The Same Parameters Of Fermenting Process, Were Obtained Yogurt Samples: A, B And C, That Are Further Analysed And Compared, By The Point Of View Of Their Physicochemical, Sensorial And Rheological Properties.

Lactic Bacteria From Commercial Starter Cultures Are Performant Strains, Selected According To Specific Criteria, With Well Defined Morphological And Physiological Characteristics.

The Aim Of This Present Paper Consists Of The Selection Of The Best Starter Culture In Order To Obtain An Yogurt With Physicochemical, Sensorial And Rheological Performances. By Applying On Laboratory Scale, The Same Conditions Of A Industrial Process, For Yogurt Production, It Could Be Relevant That The Starter Culture Have A Prominent Influence On Multiple Quality Properties Of Yogurt: Physicochemical, Sensorial And Rheological.

Results Of This Study Had Shown The Selection Criteria For The Best Starter Culture That Could Be Used In The Industrial Process For Yogurt. Correlation Between Physicochemical, Sensorial And Rheological Properties Of Yogurt Demonstrates That The Optimum Starter Culture For Yogurt Production Is That Used For Yogurt Sample C.

The Paper Highlighted The Multiple Influence Of The Quality Of Starter Cultures On The Quality Of Yogurt, As A Probiotic Product, With Large Effects On Human Health And Food Safety

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