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Potravinarstvo Slovak Journal of Food Sciences
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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 300-306
https://doi.org/10.5219/1377
Received: 4 January 2020. Accepted: 14 May 2020.
Available online: 28 May 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF COMMERCIAL YOGURT STARTER CULTURES ON FERMENTATION PROCESS, TEXTURE AND SENSORIC PARAMETERS OF WHITE YOGURT

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ABSTRACT
In this work, we have compared and described the fermentation process of two commercial yogurt starter cultures during the white yogurt production. We used freeze-dried thermophilic starter culture YoFlex® YF - L812 and deep-frozen starter culture Delvo® Fresh YS – 241 for the production of white yogurts. We analysed titration acidity, active acidity, total viable counts, texture, and sensory parameters of white yogurts produced in laboratory conditions. This research was performed for dairy company Mliekareň Kopanice Selce, s.r.o., Slovakia. We did not found statistically significant differences (p >0.05) in titration acidity of both yogurts after 7 hours of fermentation. We did not found statistically significant differences (p >0.05) in the pH of both yogurts after 7 hours of fermentation. We found statistically significant differences (p <0.05) in all textural parameters (hardness, consistency, cohesion, and viscosity). The total viable count of microorganisms in yogurts after 24 hours of fermentation was 6.28×10^7 and 7.14×10^7 respectively.

Keywords: white yogurt; fermentation; titration acidity; texture; sensory parameters

INTRODUCTION
Yogurt is commercially produced through fermentation by lactic acid bacteria (commonly *Lactobacillus* spp. and *Streptococcus* spp.) at temperatures usually in the range of 27 to 40 °C **(Aguirre-Ezkauriatza et al., 2008)**.

Yogurt is an acid-milk product characterized by symbiotic cultures of *Lactobacillus delbrueckii* subs. *Blgaricus and Streptococcus thermophilus* and senanother *Lactobacillus* species. According to the Codex Alimentarius **(CA, 2003)** and legislation of the Slovak Republic, excess of living characteristic micro-organisms in acid-milk products like yogurt and yogurt with alternative culture has to be at least 10^7 CFU.g^{-1} and if the specific microorganism is presented in the label o the product then it has to be at least 10^6 CFU.g^{-1} **(MARDSR, 2016)**.

Yogurt is one of the most popular fermented dairy products, and its consumption is increasing worldwide **(Shiby and Mishra, 2013)**.

Its popularity is due to its sensory properties, health claims, and therapeutic effects. The most important sensory attributes of yogurt include texture, color, and flavor **(Sodini et al., 2004; Salvador and Fiszman)**.

Yogurt is typically characterized as a smooth, viscous gel with a characteristic taste of sharp acid and a green apple flavor **(Cheng, 2010)**.

Interaction of taste and odor with other sensory properties increases the complexity of the human perception of yogurt. The fat content, source of milk, and textural properties play an important role in the sensory properties of yogurt **(Routry and Mishra, 2011; Mende, Rohm and Jaros, 2015)**.

The decision to change the starter culture is very important and should be supported by information. The dairy must-have information on how the fermentation will take place.

This experiment was performed specifically for dairy company Mliekareň Kopanice Selce, s.r.o (Slovakia).

Scientific hypothesis
We are expecting the significant differences in TVC, physico-chemical, and sensory parameters of yogurt produced by two commercial starter cultures YoFlex® YF - L812 and Delvo® Fresh YS – 241.

MATERIAL AND METHODOLOGY
Chemicals
All chemicals used in the experiment were analytical grade and obtained by Centralchem (Slovakia).

Yogurt starter cultures
We used two thermophilic yogurt starter cultures in our experiment:
- **YoFlex® YF – L812**, Chr. Hansen Holding A/S
- **Delvo® Fresh YS – 241**, DSM Food Specialties.

Yogurt production

Yogurts were produced under sterile conditions in our microbiology laboratory of the Department of Food Hygiene and Safety at the Faculty of Biotechnology and Food Sciences of the Slovak University of Agriculture in Nitra.

We weighed a lyophilized thermophilic frozen culture on an analytical balance (Sartorius, Genius). To prepare 30 pieces of yogurt, we used 5 liters of full 3.5% pasteurized milk and 0.2360 g of starter culture (YoFlex® YF – L812) and 0.347 g of starter culture Delvo® Fresh YS – 241. The milk was heated to 40 °C and the starter culture was then added with gently stirring 5 min. When the culture dissolved, we poured the inoculated milk into the prepared sterile 150 mL plastic cups and covered them with aluminum foil to prevent contamination. The fermentation process was carried out in a thermostat (Thermo Fisher) at temperature 32 – 33 °C (YoFlex® YF-L812) and 40 °C (Delvo® Fresh YS – 241) for 48 hours.

The amount of weighed starter culture and the fermentation temperature were chosen according to the recommendations of the producers and requirements of the dairy company.

Consequently, samples of both yogurts were analyzed at hourly intervals for period of 48 hours.

Physical-chemical analysis

- Titration acidity was determined in duplicate by titration with 0.25 N NaOH factorised with 0.25 N oxalic acid, using 2 % phenolphthalein as an indicator. Titration of milk was carried out in titration bank and yogurt in a ceramic mortar. Results were expressed in °SH (Soxhlet-Henkel method) STN (1972). Slovak Technical Standard 570530. Methods for testing of milk and milk products. Article no. 58. – Determination of titration acidity according to the Soxhlet-Henkel.
- pH (SI Analytics Lab 845 pH meter milk before coagulation and Testo 206 – pH2 after milk coagulation were used). We used calibration standard solutions with pH 6.8 and 4.0.

Microbiological analysis

The total viable count was determined by the colony-count technique and expressed as (TVC.mL^{-1}). The plates were incubated for 72 h at 30 °C.

- PCA agar with milk powder (Biokar diagnostics). Composition: (1 L of medium): tryptone 5 g, yeast extract 2.5 g, glucose 1 g, skimmed milk powder (no inhibitory substances) 1 g, bacteriological agar 12 g, pH adjusted to 7 ±0.2.
- We have followed the recommendations of ISO 7889 (2003) *Yogurt. Enumeration of characteristic microorganisms.*

Textural analysis

Textural analysis of yogurts was performed after 24 hours of the fermentation process. We used:

- Texturomenter TA.XT Plus (Stable Micro Systems UK).
- Exponent software (Stable Micro Systems).
- Disc-shaped probe with back extrusion.
- The analysis of the textural properties of yogurt sample s was performed at 6 °C.
- The instrument was set up according to the manufacturer's recommendations: mode (a measurement of compression power), options (return to start), pre-test probe speed (1.0mm.s^{-1}), test probe speed (1.0mm.s^{-1}), return speed (10.0mm.s^{-1}), deep of probe penetration into the product (30 mm), Method of measurement (Auto – 10 g), reset mode (auto), frequency of data acquisition (400 pps), the test probe was cleaned after each measurement.

Sensory analysis

Sensory analysis was performed by a sensory panel consisting of twelve assessors (7 men, 5 women) after 24 hours of the fermentation process. The evaluators were first trained. The sensory panel analyzed the samples administered in the sensory laboratory.

- We used the scale and profile method.
- We used a hedonic scale where the value of 1 indicated that the quality of the yogurt was very poor and the value of 7 was excellent.
- We chose the classic profile, which consists of creating a rough profile, then reducing the descriptors were to eliminate hedonic terms (pleasant, tasty, good), quantitative terms (too much, weak, strong), inappropriate terms (salty for smell). We chose an intensity frequency from 0 to 5, where 0 was absent and 5 very strong. Subsequently, we fine-tune the fine profile, which consisted of an overall appearance, texture in the mouth, and taste.
- Then we set the polarity of the descriptors to positive and negative, which indicates the severity coefficient. The value for these coefficients is 1. We calculated the values using the pattern descriptor intensity x severity coefficient. We get both positive and negative numbers, from which we calculated the arithmetic mean and then visualized graphically.

Statistical analysis

Statistical analysis was performed in XLSTAT 2020.1 (Addinsoft).

- Physical-chemical and microbiological analysis: we used the Shapiro-Wilkov W test and t-test.
- Textural analysis: we used the Shapiro-Wilkov W test, F-test, t-test.

We considered the results to be statistically significantly different at the α 0.05.

RESULTS AND DISCUSSION

The development of titration acidity in white yogurts during the fermentation process is presented in the **Figure 1**. We did not found statistically significant differences (p >0.05) in titration acidity of both yogurts after 7 h of fermentation. A titration acid 24 °SH was reached after 7 h of fermentation and 29 °SH were reached after 24 hours of fermentation in both yogurts.

The results of pH development during the fermentation process are presented in **Figure 2**. We did not found statistically significant differences (p >0.05) in the pH of both white yogurts after 7 h of fermentation. The pH values were 4.71 (Delvo® Fresh YS-41) and 4.86 (YoFlex YF-L812). After 24 hours the pH values were 4.46 (YoFlex YF-L812) and 4.44 (Delvo® Fresh YS-41). When we compare

the values of pH, the highest decrease was noticed during the first 6 hours of the fermentation process and that trend continued until the final control point at 48 hours after fermentation. Our results are consistent with other authors **(Tamime and Robinson, 2000; Aguirre-Ezkauriatza et al., 2008; Tomovska, Gjorgievski and Makarijoski, 2016; Akgun, Yazici and Gulec, 2017)**. The pH of commercially available yogurt was 3.94-4.22 **(Matela, Pillai and Thamae, 2019)**. The fermentation process during industrial yogurt manufacture can be continuously controlled by online monitoring of pH, which offers a useful tool for integrated process control **(Soukoulis et al., 2007)**.

The inoculated milk began to coagulate during fermentation after 5 (Delvo® Fresh YS-41) and 6 hours (YoFlex YF-L812) and pH 4.4 was reached after 15 (Delvo® Fresh YS-41) and 16 hours (YoFlex YF-L812) of fermentation in cups.

The total viable count of microorganisms in yogurts after 24 hours of fermentation is presented in **Table 1**. TVC reaches the levels 6.28×10^7 CFU.g^{-1} (YoFlex YF-L812) and 7.14 CFU.g^{-1} (Delvo® Fresh YS-41) respectively. One of the essential characteristics of yogurt is that it must contain live microorganisms used for fermentation. The total amount of living characteristic microorganisms is characterized in the mentioned legislation and is quantified as the amount of colony-forming units (CFU), which for yogurts is equal to 10^7 CFU per gram of product. In both white yogurts, the legislation limit **(CA, 2003; MARDSR, 2016)** was fulfilled after 10 hours of yogurt fermentation.

Textural parameters like hardness, adhesiveness, cohesiveness, chewiness, gumminess, springiness and sensory parameters including taste, flavor and mouthfeel, appearance, and overall acceptance are important for consumers during the shelf-life of the yogurt **(Mousavi et al., 2019)**, this was a logical reason, why we determined textural properties of both yogurths. The results of textural analysis are presented in **Table 2**. We found statistically significant differences (p <0.05) in all textural parameters and total viable counts 24 hours after fermentation. The textural parameters have reached the following mean values (YoFlex YF-L812 and Delvo® Fresh YS-41): hardness 313 and 379 g, consistency 7725 and 9990 g.s^{-1}, cohesiveness -317 and -402 g, viscosity -633 and -788 g.s^{-1}. The higher the hardness value of the yogurt, the stronger the yogurt was. We found that yogurt with Delvo® Fresh YS-241 was harder, more consistent, cohesive and viscose in comparison with YoFlex YF-L812 yogurt at selected experiment conditions, which were proposed by the dairy company. The protein matrix had an important role in cohesiveness **(Tunick, 2000)** and can be affected by the starter culture used for yogurt fermentation.

The results of sensory testing are presented in figures 3 – 8. YoFlex YF-L812 is marked as sample A and Delvo® Fresh YS-41 is marked as sample B.

According to **Figure 3,** sample A had aroma and color very good, the taste good and the consistency was satisfactory. Sample B had taste, consistency, and color very good, the smell good. The difference in taste, consistency, and flavor between both samples. According to **Figure 4** both samples had the typical yogurt flavor and acid flavor. Sample A appeared less sweet. The sensory evaluation of consistency of yogurts is presented in **Figure 5**. We can see a difference only in the softness indicator. The sensory evaluation of overall appearance is presented in **Figure 6**. Both samples were evaluated as very similar, well clothed, soft look, and thick. The sensory evaluation of texture in the mouth is presented in **Figure 7**. Sample A was evaluated as less soft and both samples were not lumpy. The sensory evaluation of taste is presented in **Figure 8**. Both your didn't differ in all taste descriptors.

Even though appearance, texture, and thickness are very important characteristics to contribute to the quality of yogurt, the flavor is generally considered as the most important of all and critical indicators of consumer acceptability **(Olugbuyiro and Oseh, 2011)**. White yogurt with a high consumer acceptance should, in general, have a smooth, uniform, and spoonable texture, and be free from lumps, graininess, and visual whey separation **(Lucey and Singh, 1997; Lucey, 2004)**. Milk heated at pH 6.7 contains significant proportions of both bound and soluble denatured whey protein complexes and this treatment produced the stiffest yogurt gels **(Ozcan, Horne and Lucey, 2015)**.

Clean and typical yogurt flavor is very important. Acetaldehyde, diacetyl, and lactic acid are considered as the major aroma components of yogurt, but also other components, like acetone, acetoin, and acetic, formic, butanoic, and propanoic acids, have been listed as contributors to yogurt flavor **(Routray and Mishra, 2011)**. Yogurt starters that allow the development of acetaldehyde with restricted post-acidification and post-proteolytic activity are favorable **(Jørgensen et al., 2019)**.

Yogurt with YoFlex YF-L812 had better flavor. On the other hand, based on the sensory evaluation we can conclude that yogurt with Delvo® Fresh YS-241 culture had slightly better consistency and taste. We identified, that the flavoring of both yogurts was better after 24 hours of fermentation in comparison with continued fermentation time. This is in agreement with **Görner and Valík (2004)**. These authors found, that formation of flavorings, in particular acetaldehyde, is mainly related to *L. delbrueckii* ssp. *bulgaricus* and is also associated with acidification. It starts at pH 5.0, increases vigorously at pH 4.4 to 4.3 in 3 h at 42 °C, then slows down significantly and stabilizes at pH 4.0.

When the pH drops below pH 5, micelles of caseins, which are amphiphilic proteins, loses its tertiary structure due to the protonation of its amino acid residues. The denatured casein proteins reassemble by interacting with other casein proteins, and these intermolecular interactions result in a network of molecules that provides the semisolid texture of yogurt **(Zourari, Accolas and Desmazeaud, 1992)**.

We agree with these authors, because, it was clearly evident, that textural parameters are developed during the syneresis process and confirmed by analysis of texture. It is not possible to analyse the texture at first stages of syneresis with the disc-shaped probe with back extrusion. We made the texture analysis 24 hours after fermentation started.

Depending on the type of yogurt, the incubation process is done either in a large tank of several hundred liters or in the final individual plastic or glass containers. The textural parameters of stirred yogurt are different in comparison with set yogurt. Stirred yogurt is fermented in the bulk tank, mixed and then poured into the final selling containers.

Set yogurt, also known as French style, is allowed to ferment right in the container it is sold in.

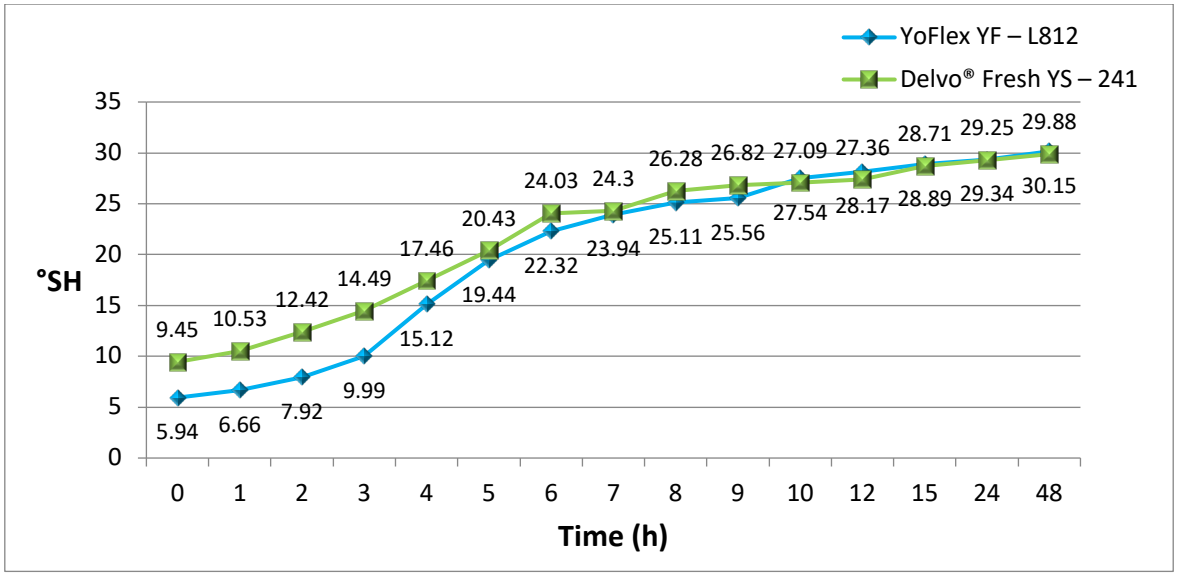

Figure 1 Development of titration acidity in white yogurts.

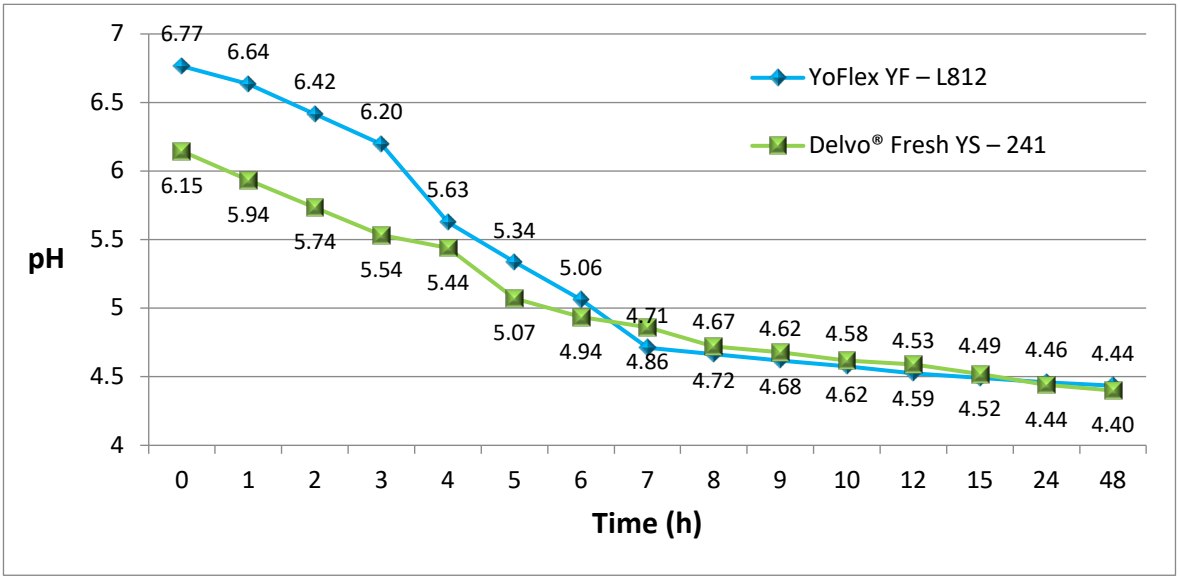

Figure 2 Development of pH in white yogurts.

Table 1 Total viable count in white yogurts (TVC.mL^{-1}).

Time (h)	Samples	
	Yogurt YoFlex® YF – L812	Yogurt Delvo® Fresh YS – 241
0 – milk with starter culter	2.16×10^2	2.18×10^2
5	3.42×10^5	2.37×10^5
10	4.81×10^7	5.21×10^7
24	6.28×10^7	7.14×10^7

Table 2 Texture properties of white yogurts.

Texture parameter	Yogurt with starter culture		
	YoFlex® YF – L812	Delvo® Fresh YS – 241	*p* value
Hardness (g)	313.118 (±38.452)	379.387 (±42.675)	0.05
Consistency (g.s^{-1})	7724.560 (±732.214)	9990.491 (±864.113)	0.05
Cohesiveness (g)	-317.405 (±39.326)	-402.404 (±49.783)	0.05
Viscosity (g.s^{-1})	-633.322 (±56.657)	-788.210 (±52.187)	0.05

Note: data in table represent the mean values and standard deviation.

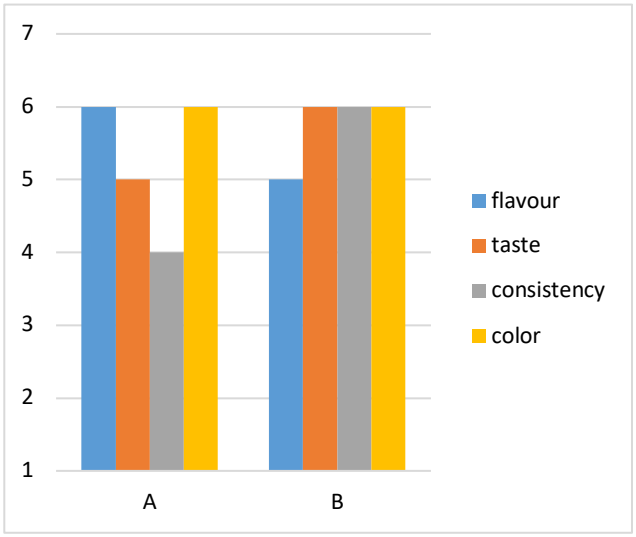

Figure 3 Sensory evaluation by quality indicator.

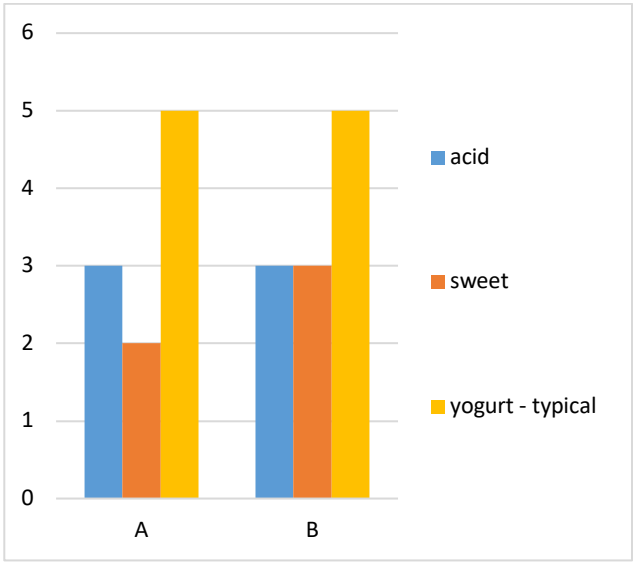

Figure 4 Sensory evaluation of flavour.

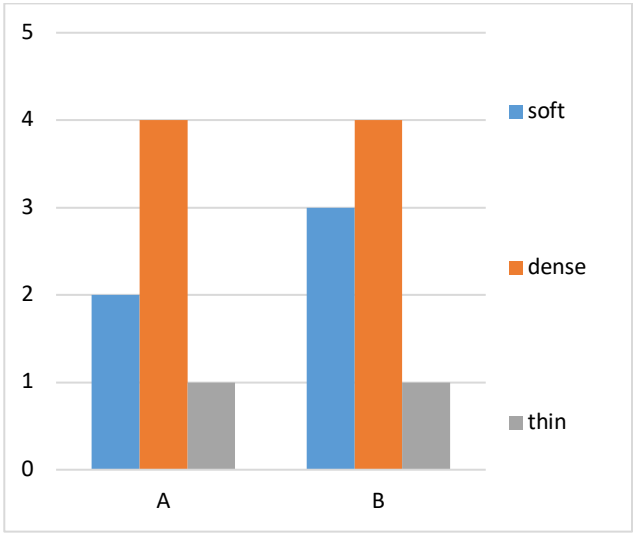

Figure 5 Sensory evaluation of consistency.

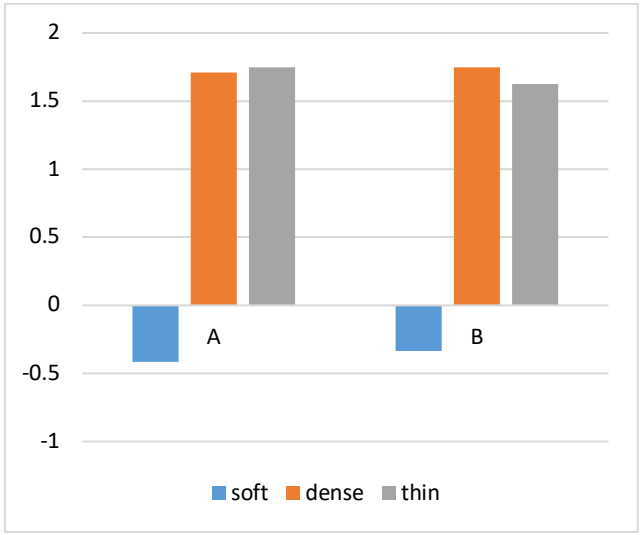

Figure 6 Sensory evaluation of overall appearance.

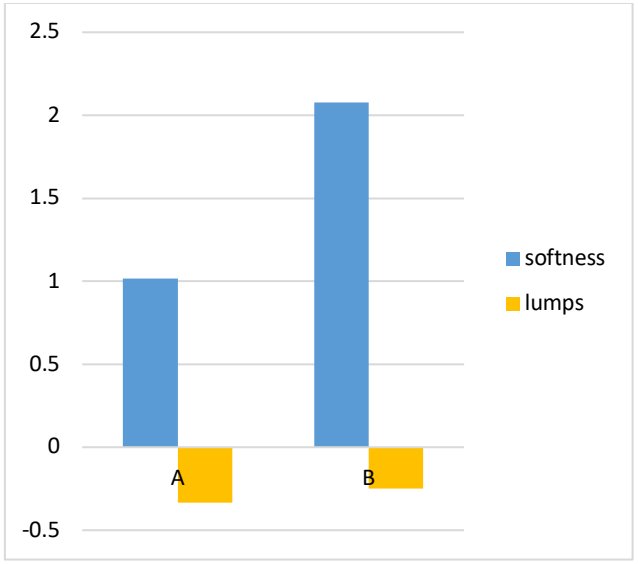

Figure 7 Sensory evaluation of texture in mounth.

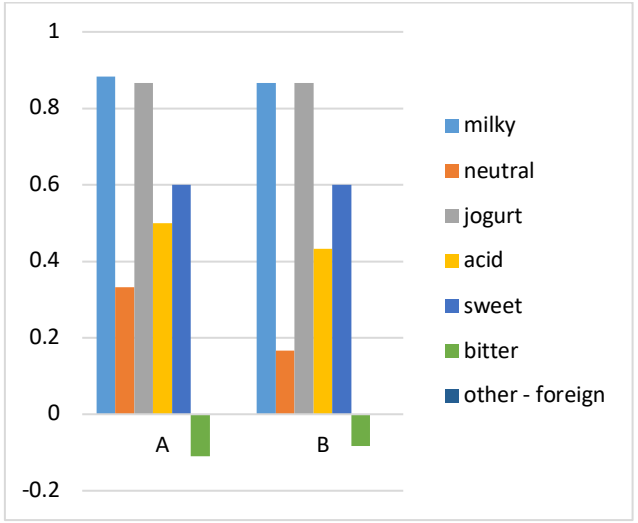

Figure 8 Sensory evaluation of taste.

In both instances, the lactic acid level is used to determine when the yogurt is ready for sale. The acid level is found by taking a sample of the product and titrating it with sodium hydroxide. A value of at least 0.9% acidity (29 °SH – 40 °SH) and a pH of about 4.4 are the current minimum standards for yogurt manufacture.

Industrial yogurt is produced by fermentation of pasteurized milk after inoculation with starter bacteria. These convert lactose to lactic acid. This changes the pH of the milk and coagulates at a certain pH. Industrial milk fermentation in yogurt production takes about 3 hours at 42 – 45 ºC. After this time, the yogurt must be cooled to a storage temperature below 10 ºC in order to interrupt the fermentation process. Durable yogurts are treated with pasteurization, which kills bacteria cultures and other microorganisms. Such treated yogurt loses biological value, mineral usability, and curative effects, especially antibacterial **(Tamime, Robinson, 1999)**.

When the yogurt reaches the desired acid level, it is cooled (10 – 12 °C), modified as necessary, and dispensed into containers (if applicable) **(Tamime and Robinson, 2000)**.

Manufacturing of yogurt has changed from crude and elementary procedures to more controlled procedures over time. A few of the advances in our knowledge that made this possible include use of ingredients in addition to milk, the use of starter cultures, and modern technologies **(Aryana and Olson, 2017)**.

CONCLUSION

Thermophilic starter cultures YoFlex® YF - L812 and Delvo® Fresh YS – 241 are suitable for yogurt production. The fermentation process using these starter cultures is slightly different. We have found, 4.4 was reached after 16 and respectively 15 hours of fermentation. We did not found statistically significant differences (p >0.05) in titration acidity of both yogurts after 7 hours of fermentation. We did not found statistically significant differences (p >0.05) in the pH of both yogurts after 7 hours of fermentation. We found statistically significant differences (p <0.05) in all textural parameters (hardness, consistency, cohesion, and viscosity). The textural parameters have reached the following values: hardness 313 and 379 g, consistency 7725 and 9990 $g.s^{-1}$, cohesion -317 and -402 g, viscosity -633 and -788 $g.s^{-1}$. The total viable count of microorganisms in yogurts after 24 hours of fermentation was 6.28×10^7 $KTJ.g^{-1}$ and 7.14 $KTJ.g^{-1}$ respectively. Based on the sensory evaluation we can conclude that yogurt with Delvo® Fresh YS-241 culture had slightly better characteristics than yogurt with YoFlex YF-L812.

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Acknowledgments:
Work was supported by the grants APVV-16-0244 "Qualitative factors affecting the production and consumption of milk and cheese".

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