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FUNCTIONAL NATURAL YOGURT TABLET BASED ON MUCILAG OKRA FRUIT AND FLEXSEED POWDER AS PREBIOTIC

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ABSTRACT

This research highlights the potential for preparing nutraceutical yogurt using okra fruit mucilage and flaxseed powder as prebiotics and the potential of probiotics (lactic bacteria) to be preserved in these prebiotics at ambient temperature. Four yogurt formulation tablets were prepared and characterized for physicochemical, biochemical, biological, and pharmacodynamic (swelling and release of bioactive substances) properties. The primary findings showed that the yogurt formulation tablets F1 based on okra fruit mucilage and flaxseed powder are rich in flavonoids and exhibit interesting activities including antioxidant activity with% of DPPH inhibition=77.263%, antibacterial and pharmacodynamic. Okra fruit mucilage and flaxseed powder are good a source of bioactive substances, which could be further used as a natural antioxidant and as a matrix for the preservation of probiotics.

1.Introduction

The intestinal microbiota is crucial for the human body with its many physiological functions, among which the digestion of unassimilated food (Bourlioux, 2014). However, the disruption of this floral can cause numerous diseases (Possemiers et al., 2009) such as inflammatory bowel disease and irritable bowel syndrome (Desscoins, 2017).

Pharmacobiotic is a strategy to address intestinal balance and prevent various affections by supplying probiotic bacteria and prebiotics (Debré et Le, 2014).

Probiotics have many beneficial therapeutic qualities, including antibacterial, anti-inflammatory, and anti-diabetic effects (Ghahfarokhi et al., 2020). It has been demonstrated that adding antioxidants from

natural sources, such as plants, is an efficient strategy to act against oxidative stress. Additionally, bioactive dietary additives can improve immune system performance (Shahein et al., 2022).

Okra (*Abelmoschus esculentus* L.) is the only important vegetable crop in the *Malvacées* family (Kumar et al., 2013). The stem, leaves, fruits, seeds, and pods of this vegetable contain important bioactive substances, including flavonoids, tannins, carotenoids, and mucilage. These substances possess dietary and therapeutic properties for diseases such as type 2 diabetes, cardiovascular disease, digestive disorders, and they can also act as antioxidants, anticancer, immunomodulators, and microbicides (Gemede et al. 2018; Belgasem et al., 2019; Elkhalifa et al., 2021). Okra mucilage has also been extensively employed in medical treatments, such as blood volume expanders and plasma replacement.

Flaxseed (*Linum usitatissimum* L.) has been grown since civilization's dawn. It has high carbohydrates content and its oil is rich in essential fatty acids in w3 (44.75%) and especially in w6 with a value of 52.69% (Benahmed Djilali et al., 2022) and protective phytochemicals that protect against chronic diseases like lignin (Madhusudhan, 2009). It has been demonstrated that flaxseed mucilages help prevent intestinal inflammation, lower blood sugar, and cholesterol. In addition, they lubricate the skin, and even take the shape of artificial mucus (Fabre et al., 2014).

According to preliminary published studies, the mucilage of okra fruit affects milk clotting. However, no research work has improved the capacity of the compact to be used as a matrix of conservation of acid lactic bacteria at ambient temperature.

This study aims to prepare a functional yogurt tablet using flaxseed powder and okra fruit mucilage as prebiotics. Some biochemical, nutraceutical, biological, and functional properties of the yogurts have been investigated.

2. Materials and methods

2.1. Plant material

Albomuschus esculentus fruit was harvested from the southern Sahara of Algeria from Ghardaia region in September 2019. Okra fruit was open-air dried (20 to 22°C) in the shade with good ventilation. The characteristics of the dried fruits included an average weight of 38.66 ± 0.94g, humidity 10%, and an acidity of roughly 3.9±0.0001%.

2.2. Biological material

Three bacterial strains (*Enterococcus feacalis*, *Staphylococcus aureus* ATCC25923 and *Staphylococcus aureus* MU50) were used to test the antibacterial effect of the ethanolic extract of the elaborated yogurt formulation, okra fruit and flaxseed. These strains were provided by the microbiology laboratory of Mouloud Mammeri University of Tizi-Ouzou.
Strains mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* from pure freeze-dried commercial yogurt (CHRISTAN HENCEN, Denmark) were employed as probiotics to prepare the yogurt.

2.3. Methods

2.3.1. Preparation of natural yogurt formulations

Several natural yogurt formulations were prepared using probiotics, prebiotics (okra fruit infused, flaxseed powder), and milk powder; four formulations were chosen (Table 1). The fermentation temperature of these yogurt formulations was kept at 45°C. Both flaxseed and milk powders were added at different concentrations to both pre-heated milk and okra fruit infusion (45°C), and each formulation was inoculated with the probiotics (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) at a concentration of 0.0015g of starter culture (each per liter). The formulation was fermented at 45°C to reach a pH of 4.5.

2.3.2. Yogurt formulation powders

The obtained yogurt (Fig 1) was spread out in a thin layer and dried for two days at 40°C using oven drying. The obtained powders were

maintained away from light and moisture until they were compressed and characterized.

Figure 1. Aspect of the elaborated yogurt formulation powders

Table 1. Composition and coagulation time of the natural yogurt formulation

Ingredients	F1	F2	F3	F4
Okra fruit infusion (mucilage) (ml)	100	100	100	50
Pasteurized milk (ml)	0	0	0	50
Flaxseed powder (g)	14	7	0	0
Milk powder (g)	0	7	14	0
Probiotics (mg)	15	15	15	15
Coagulation time (min)	75	75	75	90

The obtained yogurt powders were compacted using a HERZOG manual press using round and flat punches. Each tablet is 40mm in diameter with an average weight of 4±0.05g. 160KPa is the optimized pressure applied to obtain tablets with a tolerable level of friability.

2.3.3. Assessment methods

Phytochemical, biochemical, and rheological data were used to identify a variety of dietary and nutraceutical compounds in the elaborated yogurt formulation, including:

- ✓ Phytochemical compounds were performed according to the standard phytochemical screening methods (Kumar et al., 2018);
- ✓ Total Polyphenols Content: was quantified using Folin-Ciocalteu technique (Adrian et al., 1995) via a spectrophotometer. The absorbance at 710 nm of the two extracts (ethanolic and aqueous) for each formulation was determined. The TPC value was given as mg of Gallic Acid Equivalent per g of dry basis (mg GAE/g db);
- ✓ Total Flavonoids Content (TFC): was evaluated using a colorimetric technique (Bahorun et al., 1996). The regression equation using quercetin as the standard was utilized to determine the TFC value, which is reported as mg of Quercetin Equivalent per g of dry basis (mg QE/g db);
- ✓ The scavenging activity with the DPPH free radical (2,2-diphenyl-1-picrylhydrazyl) was employed to assess the antioxidant activity of the prepared yogurt formulation. This technique is based on evaluating the yogurt formulation to scavenge the DPPH free radical (Kim et al., 2002);
- ✓ IR spectrometry was used to analyze the functional groupings of the probiotic (lactic bacteria) to confirm the presence

of a cryoprotection agent based on bioactive substances.

3. Results and discussions

3.1. Results of the phytochemical analysis of okra fruit and flax seed

According to the phytochemical analysis, okra fruit lacked anthocyanins, free and mixed quinones, alkaloids, and sennosides. but contained gallic tannins, saponosides, flavonoids, and coumarins. Our results agree with those of Yora and Syukur, (2018) who analyzed various okra genotypes and demonstrated the absence of quinones.

Flaxseed powder is rich in flavonoids and coumarins. However, alkaloids, sennosides, free and mixed quinones, gallic tannins, and quinones were absent.

The two plant materials under investigation contain flavonoids, specifically coumarins, which are water-soluble chemicals with antioxidant, anti-inflammatory (Goyal et al., 2014) and antibacterial properties (Tiwari et al., 2016).

3.2. Results of the biochemical composition of the yogurt formulation

According to our findings (Table 2), the ethanolic extracts of yogurt formulations F1, F2, and F3 have comparable total polyphenols. Formulation F4 has a higher polyphenols concentration. Besides, significant and similar concentrations of Total Flavonoids characterize the ethanolic extracts of the elaborated yogurt F1 based on the mucilage of okra and flaxseeds have higher concentration of flavonoids with a value of 6.84±0.014 (mg QE/g d.b). This yogurt showed a very high DPPH reduction percentage compared to the other formulations, which are characterized by lower percentages.

The availability of phenolic compounds is linked to the nature of the prebiotics used (okra fruit mucilage and flaxseed) and, in part, by the composition of the cryoprotective agents of the freeze-dried lactic bacteria used as probiotics. Results show that the okra fruit mucilage and flax seeds are notable for having interesting concentrations of TP with corresponding values of 0.487 mg GAE/g d.b, 0.298 mg GAE/g d.b), respectively. Our findings are similar to other studies on the antiradical activity of plant extracts conducted by Samaniego Sanchez et al. (2007) and Liao et al. (2012).

Yogurt formulations F1 and F3 and the lactic bacteria (probiotics) can be seen to contain OH, carboxylic acids, linear hydrocarburizer group and chlorophyll by looking at the Infra-Red spectrum (Fig.2) and table 3. Peaks at 1346cm-1, 1577cm-1, and 1716 cm-1 indicate the existence of Galacturonic Acid's (GalA) carboxyl group (COOH) (Kpodo et al. 2017, Benahmed Djilali et al. 2021). The signal observed at 1091cm-1 can be considered specific to the ether group which only includes the yogurt F1.

Table 2. Biochemical composition of the yogurt formulation

Ingredients	F1	F2	F3	F4
Total sugars	0	0	1.95	0
Sucrose content	0	0	1.77	0
Reducing sugars	0	0.10	0.08	0
TPC (mg GAE/g d.b) Ethanolic extract	0.2895±0.016	0.3755±0.01	0.296±0.02	0.5465±0.023
TF(mg QE/g d.b) Ethanolic extract	6.84±0.014	5.705±0.01	5.44±0.05	5.83±0.01
% Inhibition of DPPH Water extract	4.5±1.5	31.8±13.7	6.06±3.59	3.03±1.5
Ethanolic extract	77.263±13.77	39.3±12.73	30.3±4.5	40.09±14.19

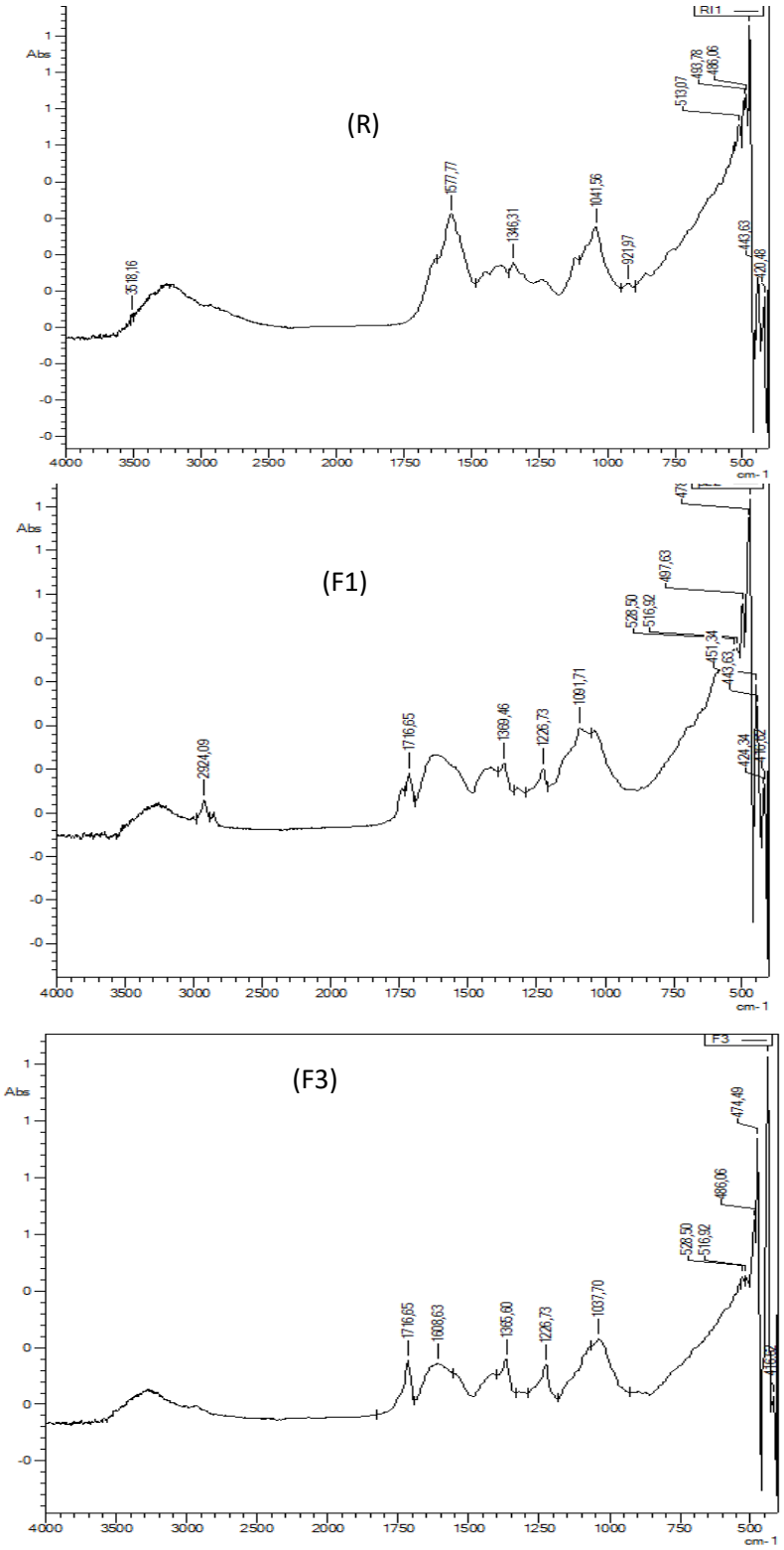

Figure 2. The Infra-Red spectrum of the probiotics (R), yogurt (F1) and (F3)

Table 3. Functional groups of yogurt formulations F1, F3 and and lactic acid bacteria (probiotics (R))

Functional groups	Yogurt F1	Yogurt F3	Probiotic (R)
Alcool (O-H)	2924 cm-1	1037 cm-1	1041 cm-1
Carboxylic (COOH)	1716 cm-1	1716 cm-1	1577-1346 cm-1
C-H	1365 cm-1	1369 cm-1	-
Amides (N-H)	1226 cm-1	1226 cm-1	-
Ether(CO-O)	1091cm-1	-	-
Amine NH2	-	-	3518 cm-1
Linear hydrocarburizer	497-478 cm-1	474-486 cm-1	493-486 cm-1
Chlorophyll	451 cm-1	416 cm-1	443-420 cm-1

3.3. Results of the antimicrobial activity of the elaborated yogurt formulation

Results of the dairy formulation's antimicrobial activity tests (Table 4) revealed that *Enterococcus feacalis* is only sensitive to the ethanolic extract of the flaxseed powder, which has an inhibition diameter of 10.66±0.02 mm. The type and amount of antioxidant nutrients, such as fatty acids, inhibit this strain. The analyzed flaxseeds oil is very rich in essential fatty acids such as linolic acid (52%), oleic acid (20.21%), and linoleic acid (15.96%). These fatty acids prevent the appearance of diseases including obesity, atherosclerosis, and some cancer (Kaleem, 2013). In addition, these acids can reduce the risk of cardiovascular disease by lowering triglyceride levels in the blood.

Entrococcus feacalis is resistant to infused okra fruit. Our findings support Solomon et al. (2016) who found that *Enterococcus feacalis* is resistant to okra flower extract.

S.aureus ATCC25923 sensitivity to the ethanolic extract of the formulation F3 with an inhibitory diameter of about 13.083mm is due to the availability of bioactive substances of okra fruit (carotenoids, mucilage, and flavonoids).

The ethanolic extracts of all formulations inhibit the *S.aureus* MU50 strain with intermediate inhibition zones. The nutraceutical compounds of okra fruit and flaxseed powder are responsible for the inhibitory activity of this strain.

Table 4. Inhibition diameters of ethanolic extracts of the different yogurt formulations, okra fruit infusion, and flaxseed powder (n=3)

Strains	**F1**	**F2**	**F3**	**F4**	**Okra fruit infusion**	**Flaxseed powder**
Enterococcus feacalis	Abs	Abs	Abs	Abs	Abs	10±0.66
***Staphylococcus aureus* ATCC25923**	Abs	Abs	13±0.083	9±0.043	9±0.001	9.3±0.005
***Staphylococcus aureus* MU50**	7±0.012	11.6±0.086	9±0.013	9±0.09	11±0.002	13±0.013

3.4. Results of some physical and rheological properties of yogurt tablet

According to the yogurt tablet formulation's rate of disintegration in distilled water (Fig 1), the tablet of formulations F1 and F2 decomposed quickly in less than 15 min. longer than 20 min, and presented low Water Holding Capacity (WHC) with a swelling index value of 4.76 and 2.66. With respect to swelling This time is based on the European Pharmacopoeia's criteria (EP, 2010). The flaxseed powder facilitates the disintegration effect.

On the other hand, both F3 and F4 yogurt tablets decomposed more slowly taking indices, formulation powders F3 and F4 exhibited higher values compared to formulation F1 and F2 with 6.33 and 5.7,

respectively. It can be explained by the herding of tablets, which is induced by the mucilage of both okra-attracting proteins and milk's calcium. Mucilage can be used as a linking agent. Our findings coincide with those of Benahmed Djilali et al. (2011) who demonstrated how date powder affects the disintegration of tablets made from a combination of date and spirulina powders.

Formulations F1 and F2 exhibit interesting rheological characteristics, including good flow, compatibility and rapid disintegration with the release of the bioactive substances.

We advise using these formulations as a therapeutic functional yogurt without sucrose, rich in prebiotics with anti-inflammatory, antioxidant, and antibacterial effects. Flaxseed powder and okra fruit mucilage can also be employed as prebiotic protection agents for probiotics (lactic bacteria).

3.4.1. SEM microstructure of yogurt

The freeze-dried lactic bacteria and the complex yogurt compositions both displayed numerous irregularly shaped, non-porous particles that are similar to polymers (Fig. 3). The yogurt formulations F1 and F2 include voluminous particles and interesting porosity between the particles, whereas F3 and F4 contain high degrees of agglomeration and fewer holes between the particles. The pores affected the disintegration and release of bioactive substances.

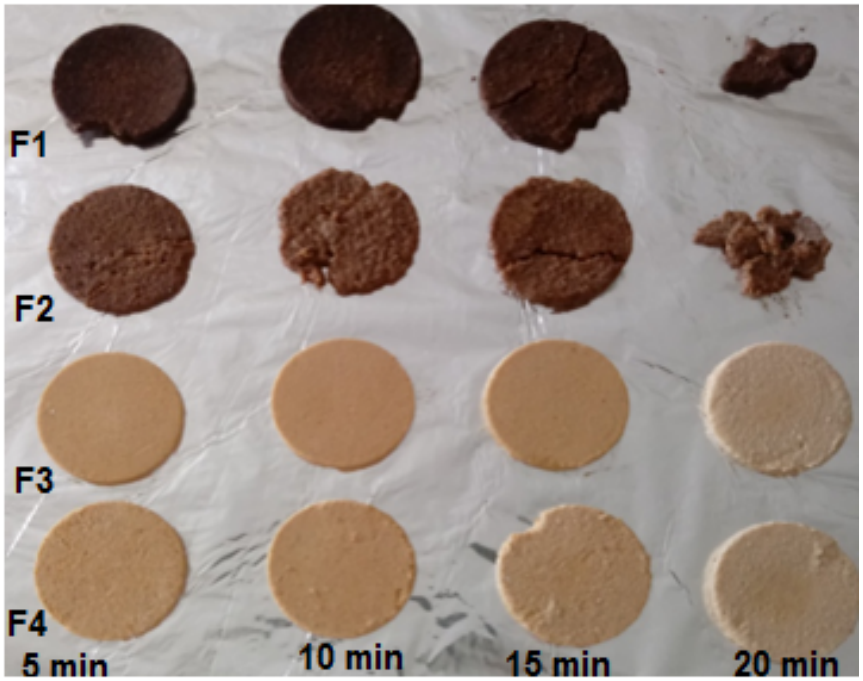

Figure 3. Morphological aspect of yogurt tablets as function of time of swelling in distilled water

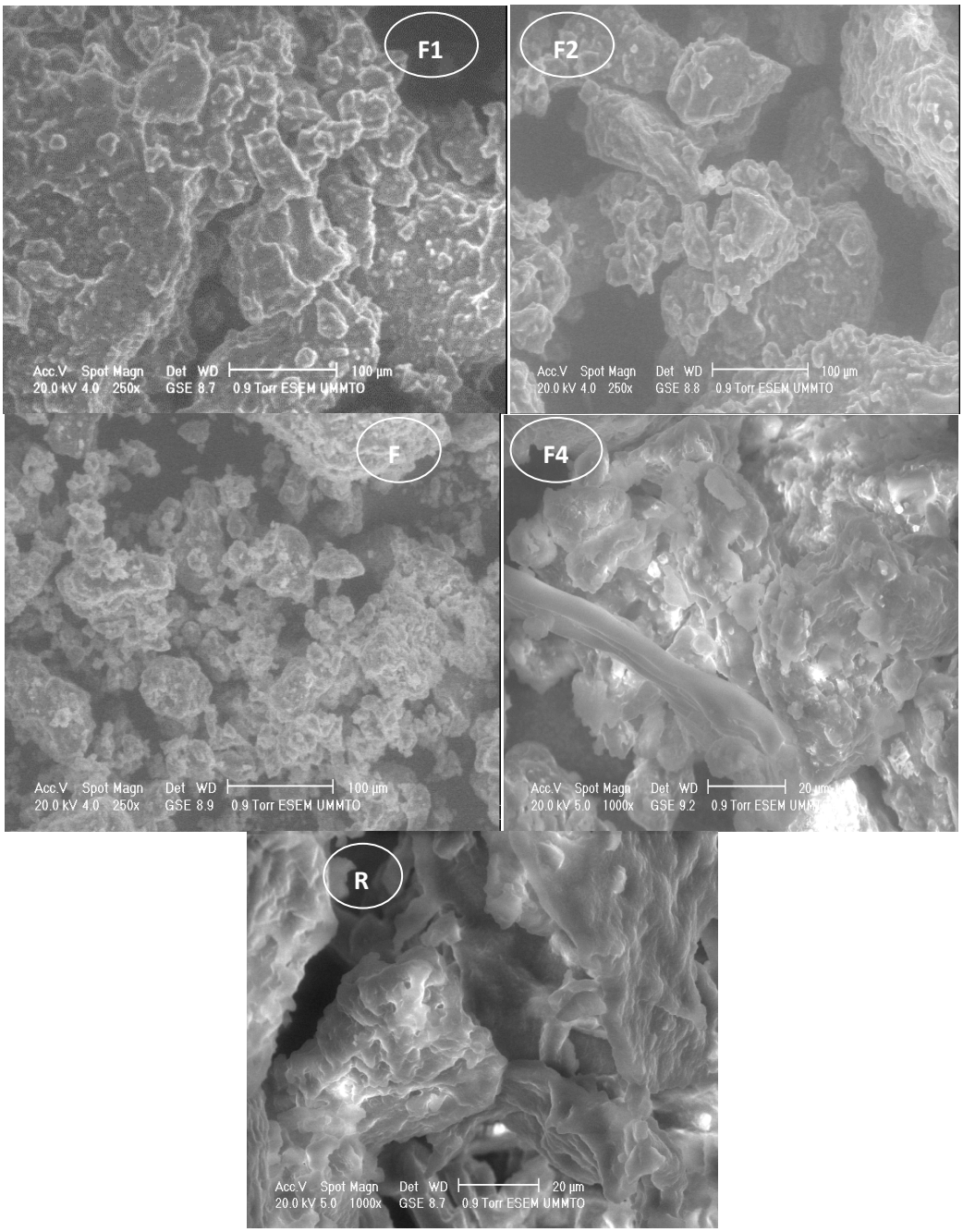

Figure 4. Microstructures of the elaborated yogurt formulations (F1, F2, F3 and F4) and lactic acid bacteria (R)

3.4.2. Stability and functionality of lactic bacteria In order to assess the viability and functionality of lactic acid bacteria, yogurt tablets stored at 30°C for three months were used to perform milk coagulation.

The F3 tablets allowed better milk clotting time (90 min) in comparison to other yogurt tablets with a time greater than 120 min.

Figure 5 shows the obtained sour milk using F1 and F3. The combination of flaxseed powder and okra fruit mucilage must be optimized to reduce the milk coagulation time

of speed. This study is currently being published.

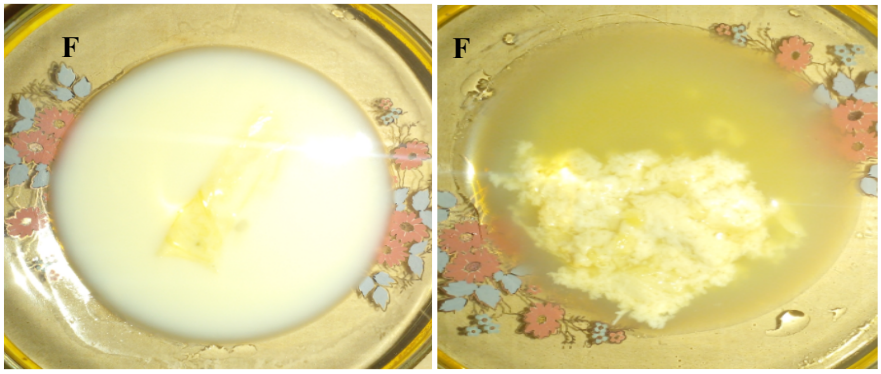

Figure 5. Obtained sour milk using tablets of yogurt F1 and F3 stored at 30°C for three months

4. Conclusions

The current study merely marks the beginning of a thorough inquiry into the medicinal and practical benefits of flaxseed powder and okra fruit mucilage, which is abundant in nutraceuticals (flavonoids and mucilage) that allow a variety of industrial uses such as agent linking, clotting milk and agent of conservation of lactic bacteria.

Further research will be required to draw additional scientific and technological information and define and apply the elaborated yogurt as a functional and therapeutic dairy product.

5. References

Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Vasseur, J., Gazin, J.C., Pinkas M., Luyckx, M., and Gazin, M. (1996). Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimttel-forschung and Drug Research*, 46 (11), 1086.

Belgasem, A., Alqamoudy, H., Shaeroun, A., Rashed, A., EL-Mahmoudy., Misbah, A.A., Almunir, N., Elshili, M., FadiaTaufik, M., Kushlaf, N. (2019). Phytochemical Screening of Okra plant from Libya. *Scholars International Journal of Chemistry and Material Sciences*. 2(6): 101-103.

Benahmed Djilali, A., Benseddik A., Boughellout H., Allaf K., Nabiev M. (2021). Biological and functional properties of vine leaves. *The North African Journal of Food and Nutrition Research*, 5 (11), 43-52.

Benahmed Djilali, A., Benseddik, A., Metahri, M.S., Lahouazi, D., Simoud, D., Nabiev, M., Allaf, K. (2022). Elaboration of New Functional Dairy Dessert Based on Flaxseed Powder, in Proceedings of the 1st International Vol 14 (4) 2022

Bourlioux, P. (2014). Actualité du microbiote intestinal, annales pharmaceutique francaises,72(1), 15-21.

Debré, P., Le Gall, J. Y. (2014). The gut microbienne, National Medical Academy, 198(9), 1667-1684.

Desscoins, L. (2017). Brain microbiota and psychiatric and neurological disorders are related. Doctoral thesis. Third University of Toulouse Peter Sabatie.

Elkhalifa, A.O., Alshammari, E., Adnan, M., Alcantara, J.C., Awadelkareem, A.M., Eltoum, N.E., Mehmood, K.H., Panda, B.P., Ashraf, S.A. (2021). Okra (*Abelmoschus esculentus*) as a potential dietary medicine with nutraceutical

importance for sustainable health applications. *Molecules*, 26(3), 696.

Ghahfarokhi MG, Yousefvand A, Gavlighi HA, Zarei M, and Farhangnia P. Developing Novel Synbiotic Low-Fat Yogurt with Fucoxylogalacturonan from Tragacanth Gum: Investigation of Quality Parameters and *Lactobacillus* Casei Survival. *Food Science & Nutrition*, 2020;8(8):4491-4504

Goyal, A., Sharma, V., Upadhyay, N., Gill, S., Sihag, M. (2014). Flax and flaxseed oil: an ancient medicine & modern functional food, *Journal of Food Science and Technology*, 51(9), 1633–1653.

Kaleem, M. (2013). Effects of products oxidation of linoleic acid on ruminal biohydrogenation. Doctoral Thesis from Toulous Univrsity, France.

Kim, J.K., Noh, J.H., Lee, S., Choi, J.S., Suh, H., Chung, H.Y., Song, Y.O., Choi, W.C. (2002). The first synthesis of 2, 3, 6-tribromo-4, 5-dihydroxybenzyl methyl ether (TDB) and its antioxidant activity. *Bulletin Korean Chemical Society*, 23(5): 661-662.

Kpodo, F.M., Agbenorhevi, J.K., Alba, K., Bingham, R.J., Oduro, I.N., Morris, G.A., Kontogiorgos, V. (2017). Kontogiorgos. Pectin isolation and characterization from six okra genotypes. *Food Hydrocolloids*, 72, 323-330.

Kumar, S., Tony, E., Kumar,P ., Kumar,A., Rao, B.S., Nadendla, R. (2013). Abelmoschus Esculentus (OKRA), *International Research Journal of Pharmaceutical and Applied Sciences*, 3(4):129-132.

Kumar, S.H.R., Das Loveleena, D., Godwin, S. (2018). Medicinal Property of Murraya Koenigii- A Review, *International Research Journal of Biological Sciences*, 2(9), 80-83.

Liao, H., Dong, W., Shi, X., Liu, H., Yuan, K. (2012). Analysis and comparison of the active components and antioxidant activities of extracts from Abelmoschus esculentus L. *Pharmacognosy Magazine*, 8(30): 156-161

Madhusudhan, B. (2009). Potential Benefi ts of Flaxseed in Health and Disease - *A Perspective, Agriculturae Conspectus Scientifics*, 74 (2),67-72.

Online Conference on Agriculture - Advances in Agricultural Science and Technology, 10–25 February 2022, MDPI: Basel, Switzerland, doi:10.3390/IOCAG2022-12266

Possemiers, S., C., Grootaert, J., Vermeiren, G., Gross, M., Marzorati, W., Verstraete, T., Wiele, V. (2009). The intestinal environment in health and disease - recent insights on the potential of intestinal bacteria to influence human health. *Current Pharmaceutical Design*, 15(18): 2051-65.

Samaniego-Sanchez, C., Gonzalez, A.M.T., Garcia-Parrilla, M. C., Granados, J.J.Q., Garcia de la Serrana, H. L., Martinez, M. C. L, (2007). Different radical scavenging tests in virgin olive oil and their relation to the total phenol conten. *Analytica Chimica Acta*. 593: 103-107.

Shahein, M.R., Atwaa, E.S.H., Babalghith, A.O., Alrashdi, B., Radwan Umair., H.A. (2022) Impact of Incorporating the Aqueous Extract of Hawthorn (*C. oxyanatha*) Leaves on Yogurt Properties and Its Therapeutic Effects against Oxidative Stress Induced by Carbon Tetrachloride in Rats. *Fermentation*; 8(5),200.

Solomon, S., Muruganantham, N., Senthamilselvi, M.M. (2016). Antimicrobial activity of Abelmoschus esculentus (flowers), *International Journal of Herbal Medicine*, 4(6), 46-49.

Yora, M. & Syukur, M. (2018). Characterization of phytochemicals and yield components in various okra (*Abelmoschus esculentus*) genotypes, *Biodiversitas*, 19 (6), 2323-2328