# <sup>1</sup>Research on the potential of postbiotics in soaps for improving their quality and functionality

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Postbiotics are metabolites of probiotic microorganisms that, unlike probiotics, do not contain live cells. This gives them greater stability and safety, making them promising for use in soap formulations. Postbiotics can enrich soaps with new functionalities, e.g., supporting the maintenance of the skin microbiome, improving hydration levels and strengthening the skin's barrier function. The present study examines their compatibility with soap ingredients and their potential as innovative cosmetic ingredients. The study aims to investigate how postbiotics can provide soaps with additional skin benefits, positioning them as innovative products in the personal care market.

Keywords: postbiotics, soaps, skin benefits

# INTRODUCTION

In recent years, the personal care industry has significant changes in consumer preferences. There is a growing preference for products with high-quality, environmentally sustainable ingredients. In response to these demands, companies are developing innovative solutions, incorporating biodegradable packaging, organic ingredients and adopting ethical production standards. this Within postbiotics are emerging as a valuable and promising cosmetic ingredient.

Soap formulation presents a challenge when incorporating active ingredients due to the high pH and the need for chemical stability and ingredient compatibility. However, to achieve the desired functionality and market appeal of soaps, various additives are used, including emollients, moisturizers, antibacterial agents and bioactive compounds [1–11].

Postbiotics also have the potential to be incorporated as an effective and innovative ingredient in soaps. They are non-living microorganisms and/or their metabolites that provide health benefits to the host [12]. These ingredients do not require viability, exhibit greater stability under various conditions, and are not subject to the same regulatory requirements for microbiological safety as probiotics, according to Cosmetics Regulation No 1223:2009 [13] and ISO 17516:2014 [14].

With the growing understanding of the skin

microbiome, postbiotics are establishing themselves as a valuable resource in cosmetics [15–18]. They contain metabolites such as short-chain fatty acids, exopolysaccharides, vitamins, teichoic acids, bacteriocins, enzymes, peptides, etc. [12, 19–21], which are used in the form of lysates, enzymes, extracts, and others [22, 23]. Postbiotics exhibit antioxidant, anti-inflammatory and immunemodulatory properties [18, 24–29], they have a longer shelf life and do not require viability in the topical formula, making them more stable and convenient for use in cosmetic products.

Their mechanisms of action can be direct (affecting skin cells) or indirect (stimulating beneficial microorganisms and inhibiting pathogens) [16]. They contribute to creating conditions that can limit skin infections and inflammation [30–34], support the treatment of acne, eczema and rosacea [15, 35, 36] and help improve skin hydration and barrier function [37–40].

The aim of this study is to examine the potential applications of postbiotics in soaps, with a focus on improving their functionality and impact on the skin. The research focuses on the effect of using postbiotic-enriched soaps on skin hydration and strengthening its microbial defenses.

The study used a postbiotic derived from *Limosilactobacillus reuteri*, a microorganism known for its ability to produce reuterin – a potent antimicrobial metabolite that effectively inhibits various pathogens [41–44]. In addition to reuterin, certain strains of *L. reuteri* are also sources of other antimicrobial compounds, including lactic acid,

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acetic acid, ethanol, and reutericyclin [45]. Among the other bioactive substances, characteristic of this species are compounds that help restore skin balance and reduce inflammation [45–47]. The stability of the postbiotic across a wide pH range, combined with its beneficial properties, makes it a suitable functional ingredient for inclusion in soaps.

# MATERIALS AND METHODS

#### Materials

The studies were conducted on solid and liquid soaps produced using a cold method, with postbiotics at different concentrations. A probiotic strain of *L. reuteri* was used, provided by the private collection of Cryobiotica Ltd.

Culture medium for obtaining the postbiotic concentrate: A solution of skimmed milk powder was prepared and whey protein was added until a concentration of 50% was reached. The resulting solution was then diluted to 15%, and 0.45% of yeast extract was added. The prepared culture medium was sterilized at 121°C for 20 min.

The formulation of the solid soap included palm oil, coconut oil (N. Pavlos, S. A. Pettas, Greece) and varying concentrations of *L. reuteri*. The saponification agent used was NaOH in the form of microbeads with a concentration of 98-99% (manufacturer: INOVYN Europe Limited). For the liquid soap, coconut oil and varying concentrations of *L. reuteri* postbiotic were used. The saponification agent used was 90%KOH in flakes (importer: Safik-Alkan Himsnab AD; manufacturer: SPOLCHEMIE, Czech Republic).

#### Methods

The probiotic microorganism *L. reuteri* was cultured in a medium for obtaining postbiotic concentrates at 37°C for 16-18 hours. The resulting culture medium was heated to 45°C, homogenized in a batch homogenizer at 400 bar, and then lyophilized in a Biobase BK-FD18P freeze dryer for 24 hours. The culture medium was previously frozen to -42 °C. Lyophilization was carried out at a working pressure of 20 Pa. The final product contains 1-4% of water.

The saponification of the oil mixture for the solid soaps was carried out in laboratory conditions using sodium hydroxide, following the method described in [48]. The appropriate amount of postbiotic concentrate was added to the soap mass. After thorough mixing, the soap mixture was poured into silicone molds and stored at room temperature (25°C) for a period of three to four weeks.

A formulation for the production of liquid soaps with coconut oil and *L. reuteri* postbiotic was also developed. The saponification of the oil mixtures

was performed in laboratory conditions. The liquid soap mass was cooled to a temperature of  $20\pm2^{\circ}C$  for 24 hours and then dispensed into bottles. A control sample (without postbiotic) was also prepared for analysis.

The soap analysis included the determination of the following physical and chemical parameters: moisture content (ISO 672:1978) [49]; free caustic alkali content (ISO 456:1973) [50]; total fatty matter content (ISO 685:2020) [51]; foaming ability (ISO 696:1975) [52]; pH (ISO 4316:1977) [53] and sensory characteristics. The sensory analysis involved evaluating the opinions of the panelists after the application of the products. The test was designed to compare the effects of different soaps (solid and liquid, with and without postbiotics) on the skin. It aimed to examine how these differences affect key indicators such as skin hydration, sensation of softness, lack of irritation, as well as foaming, hardness, soap durability and overall perception after product use.

The consumer test involved 30 panelists with healthy skin, aged between 25 and 60 years, 70% of whom were women and 60% having normal skin (as determined through self-assessment). The test was conducted in home conditions over a period of 30 days, with each product being used twice daily – in the morning and evening – for washing the hands.

Hydration was measured through the subjective feeling of moisture retention in the skin after each use. This indicator reflects how the skin feels after applying the soap – whether it is smooth, soft and comfortable, or dry and tight. The absence of irritation was assessed by observing signs of redness, itching, or other skin reactions. Foaming was evaluated based on the quantity and density of the foam, while hardness and durability referred to the soap's endurance during use and its ability to The overall perception maintain its shape. summarized the ratings for all of these characteristics.

Data were collected through surveys completed after the use of each product, with ratings given on a scale from 1 to 5. This methodology ensured a detailed and objective evaluation of the products based on real consumer experiences.

## RESULTS AND DISCUSSION

Tables 1 and 2 present the results of the analysis of solid and liquid soaps, respectively. №1A and №2A are control samples (without postbiotic), while samples №1B, №2B, №1C and №2C contain 0.1% and 0.2% of postbiotic preparation, respectively.

The results indicated that the addition of L. reuteri postbiotics to solid soaps affects some of

their physicochemical properties. The foaming capacity decreased by approximately 6-7%, which could be due to interactions between the postbiotic and surfactant components. The moisture content in the samples ranged between 18.1% and 20.4%, with the postbiotic-containing samples retaining more moisture compared to the control. This suggests a potential hydrating effect of the postbiotic.

The addition of postbiotics did not significantly affect the pH or the content of free alkalis, indicating that the saponification process is complete and the stability of the product is not compromised. The fatty acid content slightly decreased with an increase in postbiotic concentration, but the differences were not significant.

In conclusion, the postbiotic can be incorporated into solid soap formulations, contributing to a potentially enhanced hydrating effect without compromising the primary characteristics of the product.

The solid soap samples have different colors, as seen in Figure 1. This is a result of the variations in the amount of postbiotic contained in them.

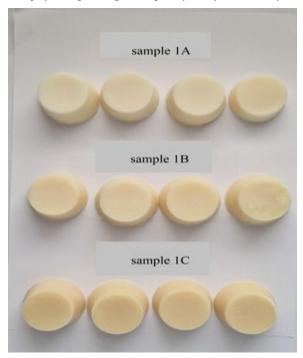


Fig. 1. Solid soap samples - (1A) control, (1B) and (1C) containing 0.1% and 0.2% postbiotic, respectively.

The darkest color is observed in sample  $\mathbb{N}_{2}$ C which contains the highest amount of postbiotic (0.2%). The control sample  $\mathbb{N}_{2}$ A stands out with a clean and lighter color.

**Table 1**. Results of the analyzed solid soap samples.

| Sample | Moisture content, % | Total fatty matter content, % | Foaming ability,<br>cm <sup>3</sup> after 30 s | рН             | Free alkali,     |
|--------|---------------------|-------------------------------|------------------------------------------------|----------------|------------------|
| №1A    | 18.1±0.1            | 61.2±0.3                      | 150.0±5.0                                      | 10.1±0.1       | $0.06 \pm 0.003$ |
| №1B    | $19.5 \pm 0.2$      | $62.3 \pm 0.3$                | $140.0\pm3.0$                                  | $10.2 \pm 0.1$ | $0.04 \pm 0.002$ |
| №1C    | $20.4 \pm 0.2$      | $63.5 \pm 0.2$                | $140.0\pm\!4.0$                                | $10.3 \pm 0.1$ | $0\pm0.004$      |

**Table 2.** Results of the analyzed liquid soap samples.

| Sample      | Free alkali,<br>% | Foaming ability,<br>cm <sup>3</sup> after 30 s | pН            | Appearance              |
|-------------|-------------------|------------------------------------------------|---------------|-------------------------|
| №2A         | 0                 | 210.0±6.0                                      | 9.2±0.1       | Transparent liquid      |
| №2B         | 0                 | $200.0\pm6.0$                                  | $9.2 \pm 0.1$ | Semi-transparent liquid |
| <b>№</b> 2C | 0                 | $180.0\pm3.0$                                  | $9.0 \pm 0.1$ | Semi-transparent liquid |

**Table 3**. Consumer test results for solid soaps

| Sample      | Hydration |      | Lack of irritation Foaming ability |      | Hardness | Overall perception |
|-------------|-----------|------|------------------------------------|------|----------|--------------------|
| №1A         | avg.      | 3.20 | 4.50                               | 4.30 | 4.60     | 3.75               |
| №1B         | avg.      | 3.60 | 4.50                               | 4.30 | 4.50     | 4.05               |
| <b>№</b> 1C | avg.      | 3.80 | 4.90                               | 4.10 | 4.60     | 4.10               |

The results of the analysis of the liquid soaps indicated that the addition of L. reuteri postbiotic leads to changes in some of their characteristics. The foaming ability decreased with an increase in the postbiotic concentration – sample N $\circ$ 2B showed a decrease of approximately 5%, while sample N $\circ$ 2C experienced a 14% reduction compared to the control sample N $\circ$ 2A.

The pH values of all samples remained within a narrow range (9.0-9.2), indicating that the addition of postbiotics does not significantly affect the alkalinity of the product. All samples have zero free alkali content, confirming the complete saponification of the oils.

A significant difference was observed in the appearance of the samples. While the control sample №2A is a transparent liquid, the postbiotic-containing samples №2B and №2C are semi-transparent (Figure 2). This indicates that the addition of postbiotics affects the optical properties of the liquid soap, likely due to the solubility or interactions of the postbiotic preparation with the other components. The difference in adding postbiotics to liquid soaps is mainly expressed in the reduction of foaming ability and the change in the appearance of the product.

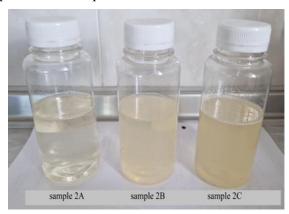


Fig. 2. Liquid soap samples - (2A) control, (2B) and (2C) containing 0.1% and 0.2% of postbiotic, respectively.

In conclusion, the addition of *L. reuteri* postbiotic to soap formulations did not lead to significant changes in the main physicochemical properties of the soap. Good compatibility with the ingredients was observed, without compromising the product's quality. This makes the postbiotic suitable for inclusion in soap formulations.

Table 4. Consumer test results for liquid soaps

The data presented in Table 3 come from consumer tests on solid soaps with postbiotics. The evaluated indicators determine the quality and overall perception of the soap by users. These factors play an important role in product evaluation, as they reflect both functional and aesthetic characteristics, which are significant to the end consumer.

Participants noted a difference in the moisture feeling after using soaps with postbiotics compared to those without. Products with higher concentrations of postbiotics demonstrated better ability to keep the skin smooth and soft after use. Sample №1C showed the highest value (3.80), indicating better hydration compared to samples №1A (3.20) and №1B (3.60). This suggests that the postbiotic in sample №1C may have a stronger effect on skin hydration.

Perceptions of irritation were relatively consistent for all samples (between 4.50 and 4.90), indicating that the addition of postbiotic does not lead to skin irritation. None of the products caused redness or itching in participants. This shows good tolerance for all tested soaps.

Regarding the foaming ability, samples №1A and №1B received the same rating (4.30), while sample №1C had a slightly lower value (4.10). This could suggest that the addition of postbiotic slightly reduces the foaming ability.

No significant changes in the hardness or erosion of the soaps were observed with the increasing concentration of postbiotic. The overall product rating combines all aspects of the consumers' perceptions regarding the soap's qualities and can be considered as an indicator of overall satisfaction with the product.

The majority of participants gave a high rating for the 'overall perception' of the soaps with postbiotics, highlighting the better feeling of hydration and skin comfort compared to the other samples.

In summary, the postbiotic has a positive effect on hydration and does not cause irritation while slightly reducing foamability, but it does not significantly impact the hardness or erosion of the soap.

Table 4 presents data from the sensory evaluation tests for the three liquid soap samples.

| Sampl | le   | Hydration Lack of irritation |      | Foaming ability | Overall perception |  |
|-------|------|------------------------------|------|-----------------|--------------------|--|
| №2A   | avg. | 3.50                         | 4.40 | 4.70            | 3.90               |  |
| №2B   | avg. | 3.60                         | 4.60 | 4.60            | 4.10               |  |
| №2C   | avg. | 3.70                         | 4.50 | 4.60            | 4.10               |  |

The participants in the test indicated that regular use of the product with postbiotics contributes to visibly better hydrated and smoother skin compared to the control sample. The products with postbiotics (№2B and №2C) received high ratings for the absence of irritation, as consumers did not experience redness or itching. The foaming ability of these products was rated as providing additional comfort and hydration.

#### CONCLUSION

Postbiotics represent an innovative and effective ingredient that can be integrated into soap formulations, opening new opportunities for development in the cosmetics industry. The results of the study show that the addition of postbiotics to soaps does not lead to unwanted changes in their core qualities, but may provide additional skin benefits, such as hydration and strengthening of the skin barrier. Although soaps are rinse-off products, the inclusion of a postbiotic suggests the potential for continued skin benefits after regular use, as a result of a cumulative effect. The use of postbiotics adds additional functionality and may contribute to successful market positioning of the products. Based on the promising results, further instrumental studies are needed to confirm the dermatological effects and the optimal conditions for the application of postbiotics in soap formulation.

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## **REFERENCES**

- O. Adigun, C. Manful, N. P. Vidal, A. Mumtaz, T. H. Pham, P. Stewart, M. Nadeem, D. Keough, R. Thomas, *Antioxidants*, 8, 536 (2019), doi: org/10.3390/antiox8110536.
- 2. A. K. Arsa, Z. Achmad, J. Techno, 7, 163 (2021).
- 3. S. Dwiyanti, S. Sulandjari, D. S. Megasari, N. Kusstanti, M. Faidah, S. Usodoningtyas, *Int. Joint Conf. Sci. Eng. (IJCSE)*, **196**, 348 (2020), doi: 10.2991/aer.k.201124.063.
- 4. H. Mawarti, M. Rajin, A. Zakaria, D. E. Purwati, *Proc. 2nd ASEAN Plus Three Graduate Research Congress (2nd AGRC)*, Bangkok, February 5-7, 2014, p. 370.
- B. T. Pamungkas, A. Safitri, F. Rezaldi, M. Andry, L. D. Agustiansyah, M. F. Fadillah, F. Hidayanto, H. Hariadi, *BIOTIK: Jurnal Ilmiah Biologi Teknologi* dan Kependidikan, 10, 179 (2022).
- Y. E. Pratama, S. Melia, E. Purwati, *Proc. IOP Conf. Ser.: Earth Environ. Sci.*, **694**, 012075 (2021), doi: 10.1088/1755-1315/694/1/012075.

- I. P. Sany, A. S. Fahmi, IOP Conf. Ser.: Earth Environ. Sci., 246, 012066 (2019), doi: 10.1088/1755-1315/246/1/012066.
- 8. S. Félix, J. Araújo, A. M. Pires, A. C. Sousa, *Waste Management*, **66**, 190 (2017).
- 9. E. D. George, D. J. Raymond, in: Soap manufacturing technology, L. Spitz (ed.), AOCS Press, 2016, p. 55.
- S. Handayani, S. Kristianingrum, A. Rakhmawati, *Orient. J. Chem.*, 34, 2410 (2018), doi: 10.13005/ojc/340524.
- 11. A. D. Susanti, S. Saputro, W. A. Wibowo, *Equilibrium J. Chem. Eng.*, **2**, 53 (2018).
- 12. S. Salminen, M. C. Collado, A. Endo, C. Hill, S. Lebeer, E. Quigley, M. E. Sandres, R. Shamir, J. R. Swann, H. Szajewska, G. Vinderola, *Nat. Rev. Gastroenterol. Hepatol.*, **18**, 649 (2021), doi: 10.1038/s41575-021-00440-6.
- 13. Regulation (EC) No 1223/2009 of the European Parliament and of the Council, of 30 November 2009, on Cosmetic Products, *Official Journal of the European Union*, **L342**, 59 (2009).
- 14. ISO 17516:2014. Cosmetics Microbiology Microbiological limits.
- 15. C. V. De Almeida, E. Antiga, M. Lulli, *Microorganisms*, **11**, 1420 (2023), doi: 10.3390/microorganisms11061420.
- A. da Silva Vale, G. V. de Melo Pereira, A. C. de Oliveira, D. P. de Carvalho Neto, L. W. Herrmann, S. G. Karp, V. T. Soccol, C. R. Soccol, Fermentation, 9, 264 (2023), doi: 10.3390/fermentation9030264.
- 17. M. Majeed, S. Majeed, K. Nagabhushanam, L. Mundkur, H. R. Rajalakshmi, K. Shah, K. Beede, *Cosmetics*, 7, 70 (2020), doi: 10.3390/cosmetics7030070.
- T. Ciardiello, D. Pinto, L. Marotta, G. Giuliani, F. Rinaldi, Cosmetics, 7, 34 (2020), doi: 10.3390/cosmetics7020034.
- B. H. Nataraj, S. A. Ali, P. V. Behare, H. Yadav, *Microb. Cell Fact.*, 19, 1 (2020), doi: 10.1186/s12934-020-01426-w.
- E. Scott, K. De Paepe, T. Van de Wiele, Biomolecules, 12, 1640 (2022), doi: 10.3390/biom12111640.
- 21. C. A. M. Wegh, S. Y. Geerlings, J. Knol, G. Roeselers, C. Belzer, *Int. J. Mol. Sci.*, **20**, 4673 (2019), doi: 10.3390/ijms20194673.
- W. Mohammedsaeed, S. Cruickshank, A. J. McBain, C. A. O'Neill, *Scientific Reports*, 5, 16147 (2015), doi: 10.1038/srep16147.
- 23. S. Puebla-Barragan, G. Reid, *Molecules*, **26**, 1249 (2021), doi: 10.3390/molecules26051249.
- 24. J. Żółkiewicz, A. Marzec, M. Ruszczyński, W. Feleszko, *Nutrients*, **12**, 2189 (2020).
- B. Liang, D. Xing, Probiotics Antimicrob. Proteins, 15, 1626 (2023), doi: 10.1007/s12602-023-10045-x.
- H. Cui, C. Feng, T. Zhang, V. Martínez-Ríos, P. Martorell, M. Tortajada, S. Cheng, S. Cheng, Z. Duan, *Sci. Rep.*, 13, 16879 (2023), doi: 10.1038/s41598-023-43336-y.
- 27. M. Duarte, A. L. Oliveira, C. Oliveira, M. Pintado, A. Amaro, A. R. Madureira, *Appl. Microbiol.*

- N. Delinska et al.: Research on the potential of postbiotics in soaps for improving their quality and functionality
- Biotechnol., **106**, 5879 (2022), doi: 10.1007/s00253-022-12116-5.
- A. Martyniak, A. Medyńska-Przęczek, A. Wędrychowicz, S. Skoczeń, P. J. Tomasik, Biomolecules, 11, 1903 (2021), doi: 10.3390/biom11121903.
- 29. E. Patil, B. Rawtal, S. Sakharwade, *World J. Pharm. Res.*, **9**, 404 (2020), doi: 10.20959/wjpr202011-18670.
- 30. M. Fournière, T. Latire, D. Souak, M. G. J. Feuilloley, G. Bedoux, *Microorganisms*, **8**, 1752 (2020), doi: 10.3390/microorganisms8111752.
- 31. S. Oh, S.-H. Kim, Y. Ko, J.-H. Sim, K. S. Kim, S.-H. Lee, S. Park, Y. J. Kim, *Food Chem. Toxicol.*, **44**, 552 (2006), doi: 10.1016/j.fct.2005.08.008.
- 32. L. Huuskonen, H. Anglenius, I. Ahonen, K. Tiihonen, *Microorganisms*, **11**, 1465 (2023), doi: org/10.3390/microorganisms11061465.
- 33. N. Golkar, Y. Ashoori, R. Heidari, N. Omidifar, S. N. Abootalebi, M. Mohkam, A. Gholami, *J. Evidence-Based Complementary Altern. Med.*, **4**, 1 (2021), doi: 10.1155/2021/8577116.
- 34. I. Castiel, L. Breton, A. Gueniche, *U.S. Patent* 10 238 897 (2019).
- 35. M. Jesenak, S. Urbancek, J. Majtan, P. Banovcin, J. Hercogova, *J. Dermatol. Treat.*, **27**, 351 (2016).
- H. Cui, C. Guo, Q. Wang, C. Feng, Z. Duan, Front. Med., 9, 1064460 (2022), doi: 10.3389/fmed.2022.1064460.
- J. Kim, Y. Lee, S. Mun, J. Jeong, D.-G. Lee, M. Kim, H. Jo, S. Lee, K. Han, J. Lee, *Int. J. Mol. Sci.*, 24, 4634 (2023), doi: 10.3390/ijms24054634.
- 38. K. M. Kim, J.-W. Song, C.-W. Lee, D.-S. Kim, J. Sohn, S. Lee, *J. Microbiol. Biotechnol.*, **34**, 65 (2023), doi: 10.4014/jmb.2306.06042.

- T. Catic, B. Pehlivanovic, N. Pljakic, A. Balicevac, *Med. Arch.*, 76, 108 (2022), doi: 10.5455/medarh.2022.76.108-114.
- 40. L.-C. Lew, M.-T. Liong, *J. Appl. Bacteriol.*, **114**, 1241 (2013), doi: 10.1111/jam.12137.
- 41. A. K. Niamah, A. A. Mohammed, N. A. Alhelf, J. *Microbiol. Biotechnol. Food Sci.*, **12**, 4701 (2023).
- 42. S. Jeznienė, PhD Thesis, KTU, Kaunas, 2024.
- 43. M.-C. Sun, Z.-Y. Hu, D.-D. Li, Y.-X. Chen, J.-H. Xi, C.-H. Zhao, *Foods*, **11**, 4000 (2022).
- 44. M. Bakhshi, S. Salari, P. G. N. Almani, S. A. K. Afshari, Gene Rep., 25, 101369 (2021).
- 45. Q. Mu, V. J. Tavella, X. M. Luo, *Front. Microbiol.*, **9**, 757 (2018).
- 46. I. Khmaladze, É. Butler, S. Fabre, J. M. Gillbro, *Exp. Dermatol.*, **28**, 822 (2019).
- 47. K. Jonas, M. Mukarram, G. Herrick, K. Frasier, V. Li, *Ameri. J. Clin. Med. Re: AJCMR 150*, **4**, (2024).
- 48. M. Perifanova-Nemska, N. Delinska, E. Dimitrova, 8th Int. Conf. Energy Efficiency and Agricultural Engineering (EE&AE), 1 (2022), doi: 10.1109/EEAE53789.2022.9831262.
- 49. ISO 672:1978. Soaps Determnation of moisture and volatile matter content Oven method.
- 50. ISO 456:1973. Surface active agents Analysis of soaps Determination of free caustic alkali.
- 51. ISO 685:2020. Analysis of soaps Determination of total alkali content and total fatty matter content.
- 52. ISO 696:1975. Surface active agents Measurement of foaming power Modified Ross-Miles method.
- ISO 4316:1977. Surface active agents Determination of pH of aqueous solutions — Potentiometric method.