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ORIGINAL ARTICLE

Investigation of the Effect of Black Pepper Powder on Microbiological and Physicochemical Properties of Processed Cheese

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KEYWORDS

Flavoring Agents;
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ABSTRACT: The recent tendency in cheese production is to produce flavored cheese using natural flavoring materials with nutritional and high-quality microbial value for human consumption. This research is aimed at investigating the microbiological and physicochemical properties of processed peppery cheese. Samples were kept under the three temperatures of 6, 25 and 37°C; microbial and physicochemical evaluations were performed on the samples immediately after being produced, after the end of each month from the production date until 4 months, and one month after the expiration date. The test was performed using a sample of processed cheese without pepper powder. The used plan was quite random, and it was repeated for 3 times in every treatment. The results of physicochemical analyses of peppery cheese compared to the control sample indicated that the peppery cheese had lower changes in pH, dry matter content and texture during the period of being kept under different temperatures and less microbial contamination was observed in it compared to the control cheese. The obtained results showed that the peppery cheese could keep the product quality better than the control cheese under different time and temperature conditions of treatments while highly controlling the changes in the chemical and microbial factors.

INTROUDUCTION

Cheese is a type of dairy product which is widely produced by using hundreds of different types of milk. Cheese is made of the coagulation of milk, usually cow, bufflehead, camel, goat and sheep milk. Milk is made sour (acidic) with the help of bacterial culture. Afterwards, it is coagulated by adding a rennet enzyme or an alternative material (such as

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acetic acid or vinegar). Whey is obtained from the curdled milk [1]. By blending the natural cheese with emulsifying salts and other dairy and non-dairy ingredients by heating and mixing them together, a processed cheese is produced which is a homogeneous product with an optimal lifetime [2, 3]. Based on the definition, production of processed cheese is divided into two main stages:

1. Selection and Formulation of ingredients:

- Selecting and crushing the natural cheese based on pH, flavor, age and healthy casein content
- Selecting healthy emulsifying salt
- Formulation and calculation of other ingredients (based on the investigation of certain properties such as: moisture, pH, fat and salt of the final product according to the state standards)

2. Processing the cheese and preserving that:

- Heating (at different temperatures and blending)
- Packing, temperature adjustment, and preservation [3, 4]

Peppers are known by of their health benefits. Plant pathologists suggest using peppers for their health-enhancing effects in long term [5]. The recent tendency in the cheese production is the production of flavored cheese using natural flavoring materials with nutritional and high-quality microbial value for human consumption [6-8]. Pepper has an antimicrobial effect on natural cheese flora, coliforms, mold, yeast, and staphylococcus aureus; it has also had an acceptable marketability [9]. Adding pepper has had a positive effect on chemical parameters including pH, moisture content, acidity and microbial parameters including total count, mold and yeast and coliform, and the lifetime of product preservation [10]. Adding different herbal extracts has had a positive effect on microbial, physicochemical and sensory quality of cheese [11]. The results of microbiological analyses suggested that pepper-flavored cheese had a good microbial quality, so that it did not contain any pathogenic bacteria, while the mold and yeast contents were also acceptable[12]. Pepper, parsley, and dill expressed an antibacterial effect on foodborne pathogenic bacteria and spoilage bacteria [13]. With regard to the recent tendency to production of flavored cheese with high nutritional value and good microbial quality, and regarding the fact that black pepper is one of the most spices with high nutritional value and quality which is widely used in Iran, as well as the increasing per capita cheese consumption, it seems that black pepper cheese can become commonly used in the household food as a new and functional product. This research is aimed at investigating the microbiological and physicochemical properties of processed peppery cheese.

MATERIAL AND METHODS

Sampling

Sampling was done in a pilot manner based on the values presented in Table 1; the samples were obtained by D-31784 Halmen (Germany) Stephan at 90 ° C and then, they were packed in the aluminum containers of Corazza FF220 DX (Italy). The pepper used in this research was purchased from Negin Khorak Pars Company with the package No. pgs2-8003 in 150-gram packs and in powder form. First, a sensory test was performed [14] on a 5-point hedonic scale in the sensory panel for different pepper percentages and the most suitable percentage was selected based on acceptability among the consumers. After the production, samples were kept under three temperatures of 6, 25 and 37°C and immediately after being produced and after the end of each month from the production date. The microbial and physicochemical evaluations were performed on the samples.

Table 1. Ingredients of processed peppery cheese.

Ingredients	Percentage
Pasteurized milk condensed by ultrafiltration	77.26
Condensate water	11.18
Butter	4
Emulsifier	2.2
Milk Protein Concentrate	1.5
Salt	1.34
Dry milk powder	1.01
Tricalcium phosphate	1.01
Pepper	0.5
Total	100

Statistical analyses

Experiments were performed in a triplicate manner. In this study, student's t-test and ANOVA were used to determine the statistical significance for pepper in the subjects classified in terms of cheese type (peppery or control). Statistical analyses were done using the statistical pack for windows, PC software, version 23 (SPSS Inc., Chicago, IL, USA). The Statistical significance was defined as $p<0.05$.

Chemical analyses

pH

After calibration and cleaning the pH meter probe (Metrohm 913), pH was measured and recorded for each sample.

Dry matter

This method is based on evaporation of water in the sample blended with sand in oven at 102 ± 2 ° C. About 20 grams of the sand was weighed in the container with glass stirring spatula. Afterwards, the container by using a glass stirring rod was heated in an oven at 102 ± 2°C for 2 hours and then, it was cooled in a desiccator to reach the ambient temperature; in the next step, it was weighed with an approximation of 0.1 mg (M1). 3 grams of the cheese sample weighed with an accuracy of 0.1 mg accuracy was transferred to the container with the sand and glass stirring rod (M2).

The cheese sample and the sand were completely mixed by using a glass stirring rod, and the mixture was transferred to the oven at 102 ± 2°C. After 6 hours, the sample container was transferred from the oven to the desiccator. It was weighed after reaching the ambient temperature (M3). The total dry matter expressed by the weight percent is equal to [15]:

$$\frac{M3-M1}{M2-M1} \times 100$$

Texture

Texture hardness of each sample was measured by using LLOYD-01 / TALS / LXE / EU and a 6-mm probe with a penetration depth of 3 mm.

Microbial analyses

Total count of microorganisms:

Using the sterile ringer's solution, a 0.1 dilution of the sample was prepared and in the sterile conditions, 1 ml of the prepared solution was incubated in a sterile plate using a sterile pipette. 15 ml of PCA culture medium with an approximate temperature of 44-47°C was added to the plate. After completely blending the culture medium with the inoculated ingredient, the plates were placed on a horizontal and cool surface to be coagulated. For controlling the sterility of the culture medium, a control plate was prepare which contained only 15 ml of culture medium. After complete coagulation of the culture medium, the plates were incubated at 30°C for 72 hours. After the end of incubation process, the colonies grown in the plate were counted using the colony counter [16].

RESULT AND DISCUSSION

pH measurement

Comparison of pH at the temperatures of 6°C (Figure 1), 25°C (Figure 2) and 37°C (Figure 3) revealed that pH decreases over time.

Although in the pepper-treated cheese sample, a pH decrease was observed during the whole 5 months, the extent of the decrease was less than the control sample as it was shown in Figure 1 - 3. A pH increase in the peppery cheese compared with the control sample can be due to adding the pepper flavor to the final applied formulation [17]. A pH decrease can finally happen due to the Millard reaction which results from a high temperature that releases organic acids out of the lactose [3]. It has been shown that a decrease in the final pH of the product could lead to a decrease in the quality of the final product [18]. Also, researchers indicated that a decrease in pH of the product might have a negative effect on its quality overtime and affects the structure of cheese texture and the ratio of proteins in the cheese [3]. Therefore, the results suggest that a lower decrease in the final pH of the peppery product over 5 months can provide a lower decrease in the quality of peppery product compared with a further decrease in pH of the control cheese; because it is protected against pH changes and according to the results obtained from a research, the peppery cheese has a higher pH stability [19]. Also according to a research, using natural materials extracted from plants were in cheese product led to higher pH stability of the final product [20]; this finding is consistent with the results of the present study

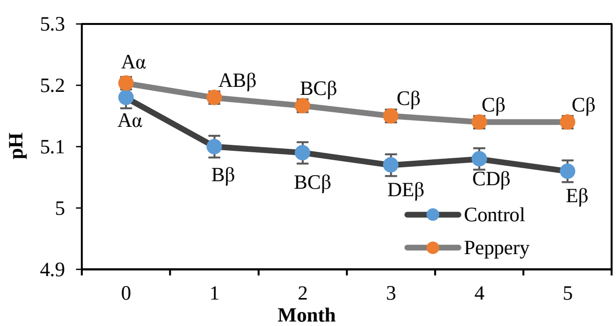

Figure 1. pH alteration trend at 6°C

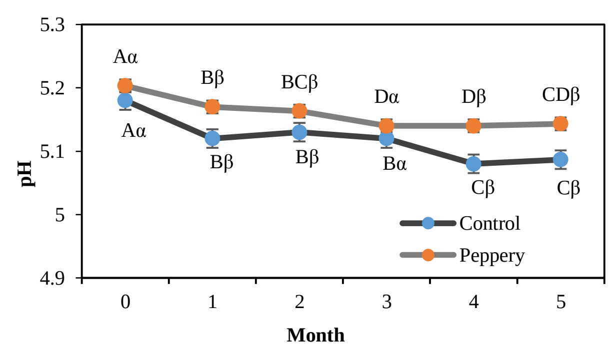

Figure 2. pH alteration trend at 25°C

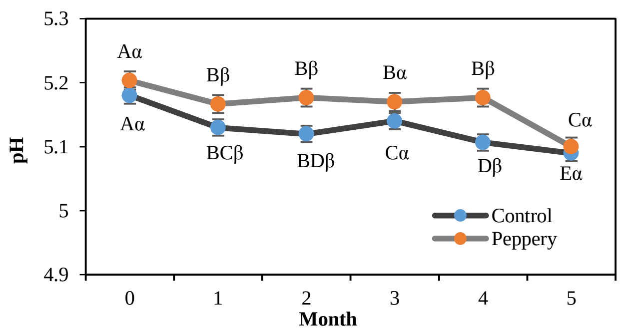

Figure 3. pH alteration trend at 37°C

Dry Matter Measurement (DM)

As observed in dry matter change diagrams (Figure 4 - 6), an increase in the dry matter content is observed in all three the temperature treatments applied during the incubation process. Moisture loss and consequently, increased dry matter content at the beginning of production was the same for all the three applied temperature treatments; but gradually this content will become greater for the temperature of 37°C (Figure 6). Furthermore, it is observed that as long as the incubation process progressed, the increase in the dry matter content for the control samples was greater than the peppery cheese samples. As the results suggest, the increase in the dry matter of the control cheese at 25 and 37°C (Figure 5, 6), was greater than the peppery cheese from the fourth month onwards and at 6°C (Figure 4); dry matter content of the control sample in the fifth month reached approximately the value of dry matter content of the peppery sample; a higher moisture loss was observed in the control sample compared with the peppery one and the peppery cheese had a better performance than the control sample in terms of maintaining its moisture.

According to the results of the studies, the gradual increase in the dry matter is due to the moisture loss in the product during the time [17, 21]. This increase in the dry matter content was higher at 37°C compared to 25°C and at 25°C compared to 6°C in both treatments of the peppery cheese and control samples. The dry matter content in the peppery cheese was higher than the control samples at 25 and 37°C, especially in the first months of the production; it can be due to adding pepper to the final product formulation and a relative increase in the dry matter content.

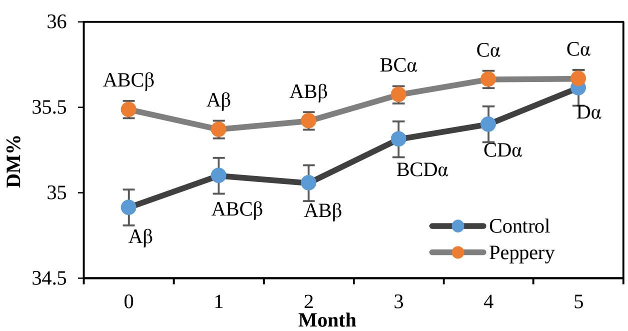

Figure 4. DM alterations trend at 6° C

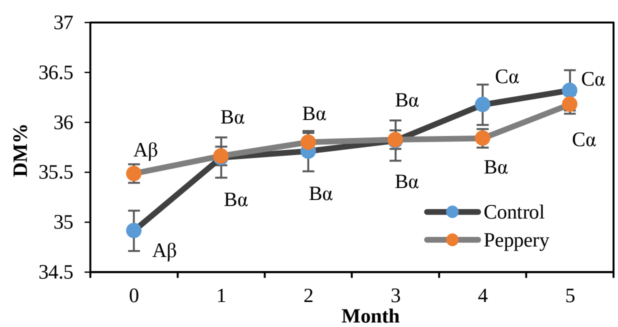

Figure 5. DM alterations trend at 25° C

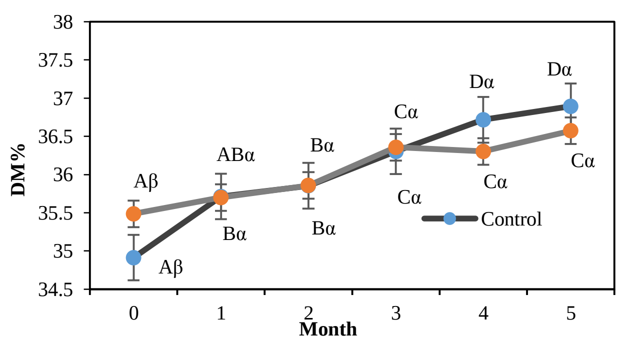

Figure 6. DM alterations trend at 37° C

Texture measurement

The results of analysis of the texture hardness in the applied temperature and time treatments suggest that the texture hardness of the cheese increased in both the peppery cheese product and the control samples over time (Figure 7-9). According to the results of analysis of the texture hardness in the applied temperature and time treatments, it can be observed that the texture hardness of the cheese, either in the peppery cheese or in the control samples, has been increased by the moisture loss in the product during the time. It can be obviously observed that the texture hardness has been significantly increased by increasing the products preservation temperature from 6°C (Figure 7) to 37°C (Figure 9). The results of dry matter content of the products are consistent with the results of product hardness resulted from decreased product moisture.

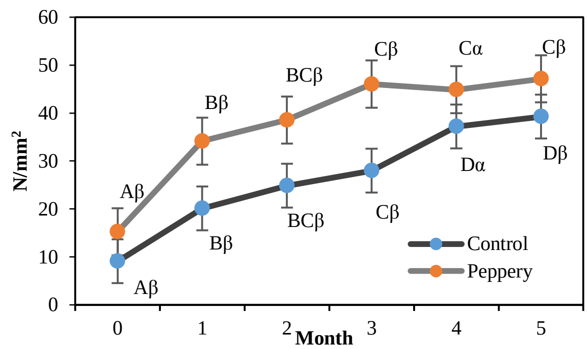

Figure7. Texture alterations trend at 6° C

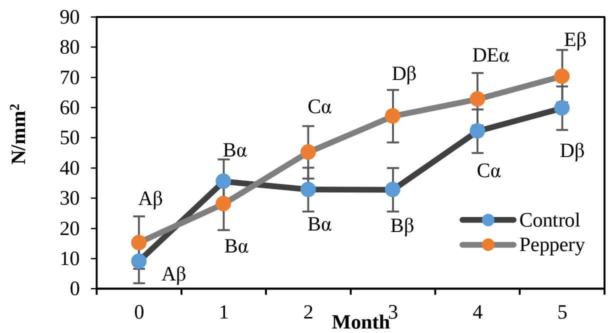

Figure 8. Texture alterations trend at 25° C

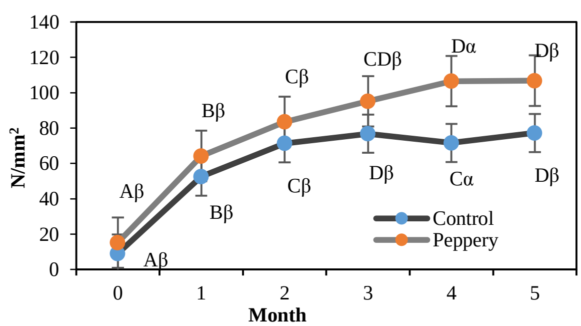

Figure 9. Texture alterations trend at 37°C

Total Count of Microorganisms

As observed in Table 2, compared with the control sample, the pepper-treated cheese has less microorganism at the three temperatures 6, 25 and 37°C.

It was also observed that the microbial count at 25°C is higher than 37 and 6°C. In the peppery cheese, this significant difference was not observed at none of the three applied temperatures.

The significant difference of temperature and time treatments between the peppery and control cheese can be caused by the antibacterial and disinfectant effects of the pepper added to the product [22]. According to the studies, in 83% of the tested cases, the effect of pepper antimicrobial activity on the tested cheese was observed which could be caused by the essential oils (EO) in the pepper[23]. Also, it has been reported that the secondary metabolites of plants can protect the product against the bacteria [24]. A higher microbial count observed at 25°C compared to 6 and 37°C (Table 2) can be due to the optimum temperature for growth of the mesophilic microorganisms at 25°C [25]. Lack of any significant difference between the peppery cheese samples treated at the three applied temperatures can be due to the pepper antimicrobial effects in the cheese.

Table 2. Microbial results table (at 95% confidence level) (cfu.gr^{-1})

Month	Control			Peppery		
	6°C	**25°C**	**37°C**	**6°C**	**25°C**	**37°C**
0	$0\pm0^{A\beta}$	$3\pm6^{A\beta}$	$3\pm6^{A\alpha}$	$20\pm10^{A\beta}$	$87\pm6^{A\beta}$	$10\pm0^{A\alpha}$
1	$0\pm0^{A\beta}$	$10\pm0^{A\beta}$	$3\pm6^{A\alpha}$	$20\pm0^{A\beta}$	$113\pm15^{B\beta}$	$20\pm0^{B\beta}$
2	$0\pm0^{A\beta}$	$7\pm12^{A\beta}$	$0\pm0^{A\beta}$	$37\pm12^{A\beta}$	$160\pm20^{C\beta}$	$30\pm0^{C\beta}$
3	$0\pm0^{A\beta}$	$10\pm17^{A\beta}$	$0\pm0^{A\beta}$	$37\pm12^{A\beta}$	$160\pm20^{C\beta}$	$33\pm6^{C\beta}$
4	$3\pm6^{A\beta}$	$13\pm12^{A\beta}$	$3\pm6^{A\beta}$	$40\pm20^{A\beta}$	$193\pm12^{D\beta}$	$40\pm0^{D\beta}$
5	$3\pm6^{A\alpha}$	$7\pm6^{A\beta}$	$7\pm12^{A\beta}$	$43\pm25^{A\alpha}$	$180\pm0^{D\beta}$	$50\pm0^{E\beta}$

CONCLUSIONS

This research attempts to investigate the physicochemical and microbial effects of black pepper powder on processed cheese. The specific issue in this study was to directly add the black pepper powder to cheese formulation. The results suggested that the antimicrobial activity of black pepper is significantly effective in reducing the total microbial count of cheese. Also, the positive effect of black pepper on physicochemical factors of cheese and relative improvement of these factors can be observed in this type of cheese. Although the sensory and market impacts of this type of pepper-flavored cheese should be investigated, these results could be considered as an evidence of higher quality and shelf life of the product; they can also be used in future studies to produce high-quality microbial and chemical flavored products.

Conflict of interests

There is no conflict of interest in the study.

REFERENCES

1. Yadav R.S., 2015. Optimization of processing parameters and extension of shelf life of ‘quark’-a type of thick yoghurt. Sunsari Technical College Journal. 2(1), 38-43.

2. Guinee T., Carić M., Kalab M., Pasteurized processed cheese and substitute. In: imitation cheese products. Cheese: Chemistry, Physics and Microbiology, Elsevier, 2004. pp. 349-394.

3. Kapoor R., Metzger L. E., 2008. Process cheese: scientific and technological aspects: a review. Comprehensive Reviews in Food Science and Food Safety. 7(2), 194-214.

4. Johnson M.E, Kapoor R, McMahon D.J, McCoy DR, Narasimmon R.G., 2009. Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. Comprehensive Reviews in Food Science and Food Safety. 8(3): 252– 68.

5. Reyes-Escogido M.D.L., Gonzalez-Mondragon E.G., Vazquez-Tzompantzi E., 2011. Chemical and pharmacological aspects of capsaicin. Molecules. 16(2), 1253-1270.

6. Foda M., El-Sheikh M., El-Kholy W., Seleet F.L., 2006. Herbs as a way for improving the quality and prolonging shelf life of soft cheese. Annals of Agricultural Science. 51(2), 457-467.

7. Hussein G., 2004. Manufactured of flavourd Tallaga cheese. Egyptian J Dairy Sci. 32, 277-290.

8. Hosny I., El Kholy W., Murad H., El Dairouty R., 2011. Antimicrobial activity of Curcumin upon pathogenic microorganisms during manufacture and storage of a novel style cheese ‘Karishcum’. Journal of American Science. 7(5), 611-618.

9. Wahba N.M., Ahmed A.S., Ebraheim Z.Z., 2010. Antimicrobial effects of pepper, parsley, and dill and their roles in the microbiological quality enhancement of

traditional Egyptian Kareish cheese. Foodborne Pathogens and Disease. 7(4), 411-418.

10. Rifky A., Shabry M., Mubarak A., 2018. Development of black pepper incorporated processed cheese spread for the local market. Development. 2(4), 6-10.

11. Gouvea F.d.S., Rosenthal A., Ferreira E. H.d.R., 2017. Plant extract and essential oils added as antimicrobials to cheeses: a review. Ciência Rural. 47(8), 1-9

12. De Souza Sora G.T., Franciosi A.E.S., Bergamasco R., Vieira A.M.S., 2013. Development and the physical, chemical, microbiological and sensory analyses of red pepper seasoned with parmesan cheese. Acta Scientiarum. Technology. 35(3), 445-450.

13. Elgayyar M., Draughon F., Golden D., Mount J., 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. Journal of Food Protection. 64(7), 1019-1024.

14. Hosseini M., Najafi H., Mohebbi M., 2013. Assessment of physico-chemical and sensory properties of imitation cheese containing whey protein concentrate and enzyme-modified Lighvan cheese. Iranian Journal of Nutrition Sciences & Food Technology. 8 (2), 91-102.

15. Iranian National Standardization Organization (I. N. S. O), 2012. NO.: 4659.

16. Iranian National Standardization Organization (I. N. S. O), 2017. NO.: 2406.

17. Masatcioglu T.M., Avsar Y.K., 2005. Effects of flavorings, storage conditions, and storage time on survival of Staphylococcus aureus in Sürk cheese. Journal of Food Protection. 68(7), 1487-1491.

18. Hamad M., Ismail M., 2013. Improvment of white cheese spread properties. Adding of some flavouring agents. Food and Dairy Sci., Mansoura Univ. 4(5), 235-245.

19. Shakerian A., Sohrabi M.J., Ghasemi Pirbalouti A., 2012. Effect of Bakhtiari celery (Kelussia odoratissma Mozaff) on sensory properties and shelf life of set yogurt. Journal of Herbal Drugs (An International Journal on Medicinal Herbs). 3(1), 41-48.

20. Shan B., Cai Y.Z., Brooks J.D., Corke H., 2011. Potential application of spice and herb extracts as natural preservatives in cheese. Journal of Medicinal Food. 14(3), 284-290.

21. Leclercq-Perlat M.N., Buono F., Lambert D., Latrille E., Spinnler H.E., Corrieu G., 2004. Controlled production of Camembert-type cheeses. Part I: Microbiological and physicochemical evolutions. Journal of Dairy Research. 71(3), 346-354.

22. Zarringhalam M., Zaringhalam J., Shadnoush M., Safaeyan F., Tekieh E., 2013. Inhibitory effect of black and red pepper and thyme extracts and essential oils on enterohemorrhagic Escherichia coli and DNase activity of Staphylococcus aureus. Iranian Journal of Pharmaceutical Research: IJPR. 12(3), 363-369.

23. Da Silva Dannenberg G., Funck G.D., Mattei F.J., Da Silva W.P., Fiorentini Â.M., 2016. Antimicrobial and antioxidant activity of essential oil from pink pepper tree (Schinus terebinthifolius Raddi) in vitro and in cheese experimentally contaminated with Listeria monocytogenes. Innovative Food Science & Emerging Technologies. 36, 120-127.

24. Tajkarimi M., Ibrahim S.A., Cliver D., 2010. Antimicrobial herb and spice compounds in food. Food Control. 21(9), 1199-1218.

25. Singh V., Das D., 2019. Potential of hydrogen production from biomass. In: Science and Engineering of Hydrogen-Based Energy Technologies. Elsevier. pp. 123-164.