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Original scientific paper

AN EFFECT OF STORAGE AND TRANSPORTATION TEMPERATURE ON QUANTITATIVE AND QUALITATIVE COMPOSITION OF MICROFLORA OF PLANT PRODUCTS

Svetlana V. Avilova¹, Vladimir N. Kornienko^{1*}, Aleksey A. Gryzunov¹, Anna A. Vankova²

¹ All-Russian Scientific Research Institute of Refrigeration Industry —

Branch of V. M. Gorbatov Federal Research Center for Food Systems of RAS, Moscow, Russia

² Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, Russia

ABSTRACT

KEY WORDS: storage, transportation, temperature regime, plant products, microorganisms, microbiological spoilage

The results of the study on changes in the composition and quantity of epiphytic and endophytic microorganisms of plant products during storage and transportation are presented. For the investigation, the authors took apple fruits and leafy spicy green products that had biological peculiarities and allowed investigating processes of the long-term and short-term main stages (cold storage, transportation by refrigerated transport, presales storage) of the continuous cold chain on the way to a consumer. Apple fruits were placed in storage in cold chambers with the temperature regimes of plus (2–3) °C and minus (1–2) °C, where they were stored for 90 days. The vegetative organs of dill and parsley were transported during 8 hours by a refrigerated truck and placed in the commercial refrigeration equipment at two temperature regimes (4-5) °C and (0-1) °C for 72 hours for presales storage. The results of the microbiological analysis showed that the number of endophytic microorganisms (bacteria, yeasts and molds) was lower by 1-3 orders of magnitude in apple fruits and by 2-3 times in green vegetables compared to the number of epiphytic microorganisms. It was established that the regime of storage at negative temperatures completely inhibited the development of epiphytic bacteria on fruits, significantly delayed the multiplication of epiphytic yeasts and molds; while at a positive temperature the number of bacteria increased approximately by 10-17 times, yeasts by 180 times and molds by 3 times. The dynamics of changes in the number of endophytic microorganisms during storage showed the same trend that was observed for epiphytic microorganisms. Analysis of the microbial quantity after transportation of green products showed an increase in abundance of the revealed groups of epiphytes and endophytes by 1.5-3 times upon absolute prevalence of bacteria. After short-term storage, a significant growth of the revealed microbial groups was found; with that, their quantity was 1.5–6.5 times higher at (4-5) °C than at (0-1) °C. The authors experimentally confirmed the conclusion that with respect to reduction of losses due to microbiological spoilage and extension of shelf life, the cold storage regime of the studied plant products at near-zero temperatures is preferable compared to the regimes of storage at higher positive temperatures.

1. Introduction

Products of plant production are necessary for the human body as a source of vitamins, carbohydrates, microelements, mineral salts, amino acids and other substances. Fruits and vegetables are consumed mainly in the fresh state; therefore, to ensure healthy and safe nutrition, it is necessary to carry out not only chemicaltoxicological but also microbiological control of products [1].

Spoilage of plant products occurs due to the natural physiological processes and microbial activity [2]. According to some estimates, a proportion of registered losses caused by the microbial activity reaches (30–40)%. Spoilage occurs throughout the food chain — during harvest, processing and storage of crops, during transportation, wholesale and retail sales up to consumption by population [3].

One of the solutions to the strategic task of reducing food losses is regulation of microbiological spoilage, which requires knowledge of the composition of the microbial community including both microorganisms typical for a certain type of plant products and spoilage microorganisms. The use of knowledge about microbial behavior and their habitat for regulation of microbiological parameters will provide producers and suppliers of fresh fruit and vegetable products with an opportunity to improve existing technologies of storage and transportation [4,5,6].

Preservation of fruits and vegetables in the fresh state is achieved due to a reduction of the vital processes both directly in plant products and in microbial community [7]. By regulating environmental conditions (temperature, humidity, gas composition, quantity of available nutrients, acidity and others), it is possible to significantly retard life processes in microorganisms increasing therewith storability of plant products, their resistance to diseases in the process of distribution from a manufacturer to a consumer [2,8].

Artificial cold that underlies all modern technologies for individual links of the continuous cold chain (CCC) allows a substantial reduction of losses due to a decrease in microbiological spoilage, maximum retardation of physiological and biochemical processes and consequently prolongation of shelf-life of fruits and vegetables without significant deterioration of their quality [9]. The CCC as an inter-branch organizational technological complex is intended for food safety and storability assurance at a high-level throughout the way from a producer to a consumer (refrigerated storage, transportation by refrigerated transport, presales storage) due to continuity of the specified thermal condition of foods [10,11].

Violation of required thermal regimes, emergence of cyclic and variable temperature effects of the environment during storage and transportation lead to a sharp acceleration of the processes of bacterial spoilage in products, which negatively affect their quality indicators and safety in a sales process [12, 13, 14, 15].

A quantity and composition of microorganisms on the surface and in internal tissues of fruits and vegetables at different ambient temperatures are studied insufficiently. Investigations in this direction are of great practical importance and create a basis for improving parameters of storage and transportation of plant products.

Fruits and vegetables intended for storage are the living vegetative and reproductive organs of plants, which surface is abun-

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dantly inhabited by microorganisms that were given the name «epiphytes» [16,17]. Recently, more and more information have appeared showing that plant internal tissues are also inhabited by microorganisms called endophytes [18,19,20,21,22].

The aim of the investigation was to study the composition and number of epiphytic and endophytic microorganisms of different types of fruit and vegetable products during storage and transportation at different temperature regimes.

2. Materials and methods

Apple fruits and leafy spicy green products were taken for the investigation. This choice was based on differences in their biological peculiarities that allowed investigating processes of the long-term and short-term main stages of the CCC on the way to a consumer.

Storability of apple fruits is largely determined by duration of a period of post-harvest ripening at corresponding temperature and humidity regimes (storage in a cold chamber). Due to low duration of transportation compared to the storage period, a temperature of transportation practically does not influence apple storability. Only mechanical damages during transportation can be the main causes of subsequent quality deterioration.

The objects of the investigation of seed fruit crops were the apple varieties «Royal Gala» and «Champion», which were placed in storage in cold chambers (with a positive temperature of plus (2-3) °C and with a negative temperature of minus (1-2) °C) and stored for 90 days.

Storability of leafy vegetables is low; their preservation almost entirely depends on external conditions upon supply to a consumer. Leafy vegetables are characterized by slight loss of water due to the highly developed leaf surface, thin cell walls and cover tissues, weak water holding capacity of colloids. Therefore, spoilage and quality deterioration of fresh green products mainly occur during the short periods of their transportation and sale [23].

The objects of investigation of green cultures were dill variety «Alligator» and parsley variety «Kharkovchanka». Freshly harvested green products were delivered (for 8 hours by refrigerated trucks) and stored before sale (for 72 hours in the retail refrigerated equipment) at two temperature regimes: (4-5) °C and (0-1) °C.

The microbial quantity and composition in apple fruits were analyzed by the standard method of plating on the glucose peptone medium (GPM) [24]. To detect yeasts, 40% lactic acid was additionally introduced into the medium. To detect epiphytic microorganisms, the surface of the preliminarily weighted apples was swabbed with sterile water. Then, the fruit surface was sterilized with 96% ethanol, the cover tissue was removed with a sterile scalpel and an average sample of internal storage tissues was taken for detection of endophytic microorganisms. The consecutive serial dilutions were prepared and pour plating on the GPM was carried out in duplicate by the conventional methods [25].

The microbial complex of green cultures upon transportation was also analyzed by the standard method of plating on the GPM. To detect epiphytic microorganisms, a specimen of an average sample was washed in sterile water in the process of agitation in a shaker for 10 min. To detect endophytes, the washed samples of green vegetables were transferred to 96% ethanol, agitated for 1 min., rinsed several times with sterile water and mechanically chopped until obtaining homogeneous mass.

3. Results and discussion

Apple fruits. As the results of the investigation showed, microorganisms (bacteria, yeasts and molds) were found both on the surface and in internal tissues.

Microbial community of epiphytic microorganisms was different on the apples of the analyzed varieties before placement in storage (Figure 1). For example, bacteria were found only on the apples of the «Champion» variety; yeasts, which are the typical inhabitants of the phyllosphere, were practically absent on the apples of both varieties, and molds were present in low numbers on the surface of the «Royal Gala» fruits (Figure 1 a, b, e).

The number of endophytic yeasts was extremely negligible on the apple fruits of both varieties and endophytic molds were absent (Figure 1 d, f). In the «Champion» variety, endophytic bacteria were found in small numbers (Figure 1b).

During storage, a quantity of the studied microbial groups changed depending on a variety and temperature conditions. At the positive temperature regime of plus (2-3) °C, the maximum number of epiphytic bacteria was revealed on the apples of the «Champion» variety. During storage, their number increased by approximately 15 times (Fig. 1a). The storage regime at negative temperatures of minus (1-2) °C completely inhibited the development of epiphytic bacteria on fruits of both varieties (Figure 1a).

The number of yeasts increased during storage with more intensive multiplication of yeasts at a positive temperature than at a negative temperature. For example, at the temperature regime of (2-3) °C, the number of yeasts increased by 180 times on the «Royal Gala» variety and by 10 times on the «Champion» variety compared to the initial values before storage; while at a temperature of minus (1-2) °C, it increased by approximately 95 times on the «Royal Gala» fruits (Fig. 1c). Therefore, a negative storage temperature did not completely inhibit the development of epiphytic yeasts on the «Royal Gala» variety but retarded their multiplication. An increase in the number of yeasts on the fruits of the «Champion» variety was observed only at a positive storage temperature and they were not revealed at a negative temperature (Figure 1c).

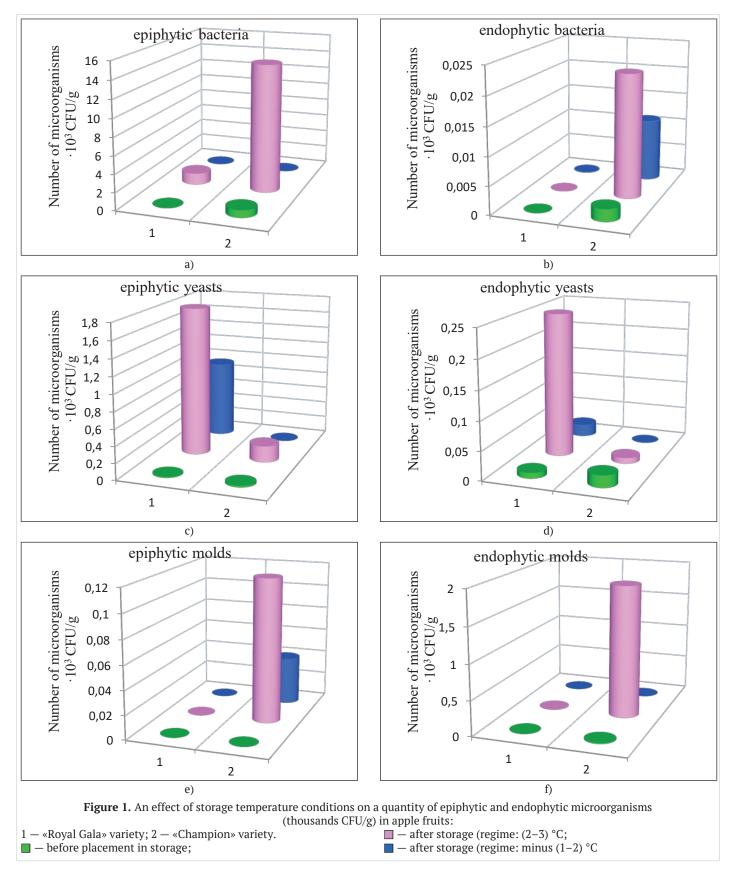
The composition of epiphytic microorganisms usually contains molds, including phytopathogenic that contaminate fruits during harvest, transportation and storage, and quickly develop upon favorable temperature and humidity conditions. However, molds were not found on the fruits upon placement of the apples of the studied varieties in storage (Figure 1 e).

During storage at the temperature regime of (2-3) °C, molds developed on the «Champion» fruits in a quantity that was three times higher than the values obtained after storage at the temperature regime of minus (1-2) °C (Figure 1 e). In the «Royal Gala» variety, molds were not revealed on the fruit surface after storage both at positive and negative temperatures (Figure 1 e).

The results of the microbiological analysis showed that the number of endophytic microorganisms was by 1–3 orders of magnitude lower than epiphytic (Figure 1 b, d, f). In the structure of the microbial community, endophytic bacteria were absent in the «Royal Gala» fruits; individual bacterial cells were found only inside apple tissues of the «Champion» variety (Figure 1b).

The dynamics of changes in the number of endophytic yeasts during storage showed the same trend that was observed for epiphytic yeasts. At a temperature of (2-3) °C, the multiplication of endophytic yeasts was more intensive: an increase by 25 times was found in the fruits of the «Royal Gala» variety. In the fruits of the «Champion» variety, yeasts were not revealed after storage at a negative temperature, while at a positive temperature, a decrease in their initial number by approximately 2 times was established (Figure 1 d).

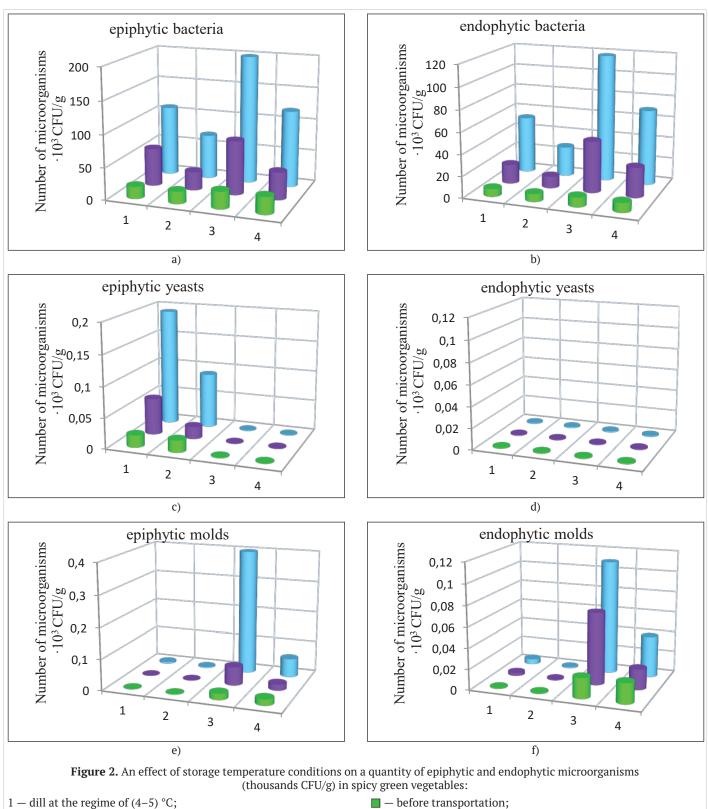
During storage of apples of the «Champion» variety at the temperature regime of (2-3) °C, a large number of molds developed in internal tissues (Figure 1f). At the same time, the development of molds was completely inhibited in the fruits of this variety at the temperature regime of minus (1-2) °C. In the fruits of the «Royal Gala» variety, the development of mold cells was not recorded during storage. Therefore, a negative ambient temperature completely stopped the growth of endophytic molds on the fruits of both varieties (Figure 1f).



Spicy leafy greens. The results of the microbiological analysis of fresh green products during transportation and the following short-term storage before sale showed that microorganisms (bacteria, yeasts and molds) were present both on the surface and in internal tissues of dill and parsley. The total microbial number changed depending on a species of the green culture.

The structure of the epiphytic microbial complex of the dill leafy apparatus before transportation was presented by bacteria and yeasts, and those of the endophytic microbial complex only by bacteria. The vegetative organs of parsley separated from plants were slightly different from dill regarding the microbial composition — bacteria and filamentous fungi were found on the surface and in internal tissues (Figure 2).

The absolute prevalence of bacteria was common for all types of green crops. Their number was a thousand times higher than the number of yeasts and molds (Figure 2 a, c, e). The initial number



2 – dill at the regime of (0-1) °C;

- 3 parsley at the regime of (4-5) °C;
- 4 parsley at the regime of (0-1) °C

of epiphytes and endophytes (Figure 2). In our opinion, this effect was associated with changes in temperature-humidity conditions

of epiphytic bacteria on freshly harvested parsley before transportation was about 1.5 times higher than on dill (Figure 2a). A quantity of epiphytic bacteria was several times higher than those of endophytic bacteria: the number of epiphytic bacteria on dill was 2.5 times higher and on parsley 3 times higher than the number of endophytic bacteria, respectively (Figure 2 a, b).

of transportation [23]. Figure 3 presents the character of changes in the air temperature in the body of a refrigerated truck and on the surface of fresh green vegetables at the transportation temperature regime of

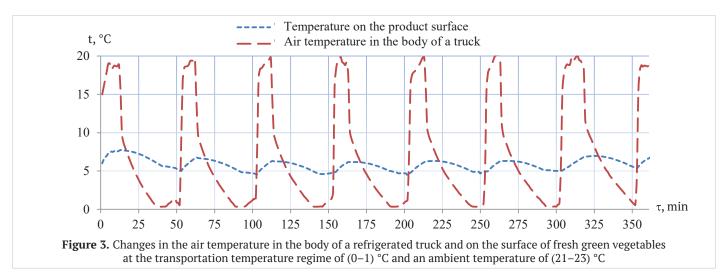
(0-1) °C. As a result of the impact of the external peak incoming heat load upon door opening during (7-10) min. for unloading

Analysis of microbial quantity after transportation of green products showed an increase in abundance of the revealed groups

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- after transportation;

— after presales storage



part of products at different points of sale, the required temperature regime of transportation of green crops was not maintained during most part (80-90%) of the transportation period; with that, a temperature on the surface of green vegetables varied in a range of (5-7) °C.

During transportation, moisture of warm ambient air entered the body of a refrigerated truck upon opening the door, which led to condensate dropout and formation of condensed moisture on the leaf surface of spicy green vegetables. The favorable conditions for penetration of microorganisms into the lamina were created. Moreover, substances from the surface of tissues dissolved in the formed water. These substances served as nutrition for microorganisms and stimulants for the development, which accelerated their penetration into internal tissues of a leaf.

As a result of the conducted experiments, it was found that a sharp growth in the microbial community of green products was not observed immediately after the end of the transportation process. For example, the number of epiphytic bacteria increased by approximately 1.5 times on dill and parsley at the temperature regime of (0-1) °C (Figure 2a). With that, the number of epiphytic yeasts on dill and epiphytic molds on parsley did not change (Figure 2 c, e). An increase in endophytic microorganisms at the temperature regime of (0-1) °C (0-1) °C was found: by approximately 1.6 times for bacteria on dill and parsley; by 3.5 times for molds on parsley (Figure 2 b, f).

At the same time, the number of epiphytic bacteria increased by approximately 3 times on dill and parsley at the temperature regime of (4-5) °C (Figure 2a). The number of epiphytic yeasts on dill and epiphytic molds on parsley also increased by 3 times (Figure 2 c, e). With that, the number of endophytic microorganisms increased as well: bacteria by approximately 2.5 times on dill and by more than 5 times on parsley; molds by 3.5 times on parsley (Figure 2 b, f). Endophytic yeasts in both types of green vegetables were absent (Fig.2 d).

However, after 3 days of presales storage in the retail refrigerated equipment upon maintaining adequate temperature regimes of storage, certain microbiological indicators of spicy green vegetables significantly deteriorated.

At the temperature regime of (4-5) °C, there was an increase in the number of epiphytic bacteria by approximately 6 times on dill and by more than 7 times on parsley, epiphytic yeasts by approximately 10 times on dill and epiphytic molds by 20 times of parsley (Figure 2 a, c, e) compared to the initial data (before transportation). The number of endophytic microorganisms also increased: bacteria by more than 7 times in dill and by approximately 13 times in parsley; molds by 5.5 times in parsley (Figure 2 b, f).

At the temperature regime of storage of (0-1) °C, the development and multiplication of the microbial community of the vegetative organs of green products were slowed down. For example, the number of epiphytic and endophytic bacteria increased by approximately 3.6 and 3.8 times in dill, by 4.4 and 7.7 times in parsley, respectively, compared to the initial data (before transportation). The number of epiphytic yeasts increased by 4.5 times on dill, the number of epiphytic and endophytic molds by 3 times and 2 times in parsley, respectively (Figure 2 c, e, f).

4. Conclusion

Based on the performed research, it can be concluded that the quantitative and species composition of the microbial community of plant products and the character of its changes depend on the temperature parameters of storage and transportation.

For apple fruits of the studied varieties that were stored at a positive temperature, the number of the revealed microbial groups increased: bacteria by approximately 10–17 times, yeasts by 95 to 180 times, molds by 3 times. At the same time, the temperature regime of minus (1–2) °C completely inhibited the activity of epiphytic bacteria (the «Champion» variety), considerably delayed the multiplication of bacteria and yeasts (the «Royal Gala» variety), and also significantly slowed down or completely stopped the growth of endophytic microorganisms. Epiphytic bacteria and endophytic molds, which cause spoilage of fruits of these varieties at positive temperatures of storage, were not revealed in the conditions of negative temperatures.

With respect to reduction of losses from microbiological spoilage, the regime of refrigerated storage of apple fruits at negative temperatures is preferable compared to the recommended regimes of storage at positive temperatures.

It is generally agreed that short-term violation of temperaturehumidity regimes has no effect on food quality indicators [26]. At the same time, variations in ambient temperature and humidity, as in the case of transportation of spicy green vegetables, can cause the formation of condensed moisture on the surface of plant products, an increase in the intensity of its breath, the development and multiplication of epiphytic and endophytic microorganisms, which, finally, can lead to loss of quality and product spoilage during following storage.

Analysis of microbial quantity after transportation of green products showed an increase in abundance of the revealed groups of epiphytes and endophytes by 1.5-3 times upon absolute prevalence of bacteria. After short-term storage, a significant growth of the revealed groups of microorganisms took place; with that, their quantity was 1.5-6.5 times higher at (4-5) °C than at (0-1) °C.

As the performed investigations show, the multiplication and development of microorganisms during transportation reduced resistance of green crops to further damage of tissues by microbiota.

Near-zero temperatures of transportation and storage reduced the growth of the microbial community during following short-term storage of green products by several times. Therefore, to maintain high quality of green crops and reduce losses, it is necessary to store and transport them at a temperature of 0 °C - 0.5 °C. When adhering to these conditions, shelf-life of green products can be significantly prolonged.

It was established that the process of the development and multiplication of epiphytic and endophytic microorganisms of

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plant products significantly depends on the temperature regimes and their variation during storage and transportation.

Detection of microbial contamination of fruits and vegetables before their movement along the main stages of the CCC will permit improving (regulating) temperature-humidity parameters of the processes of storage, transportation and presales storage of plant products, increasing their quality, reducing losses and extending shelf-life.

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AUTHOR INFORMATION

Svetlana V. Avilova - candidate of agricultural sciences, docent, leading research scientist, Laboratory of refrigeration processing and storage of food products, All-Russian Scientific Research Institute of Refrigeration Industry - Branch of V. M. Gorbatov Federal Research Center for Food Systems of the RAS, 127422, Moscow, Kostyakova str., 12. Tel.: +7-495-610-80-92, e-mail: sv.avilova@mail.ru

Vladimir N. Kornienko – candidate of technical sciences, docent, leading research scientist, Laboratory of refrigeration processing and storage of food products, All-Russian Scientific Research Institute of Refrigeration Industry – Branch of V. M. Gorbatov Federal Research Center for Food Systems of the RAS, 127422, Moscow, Kostyakova str., 12. Tel.: +7–495–610–80–92, e-mail: kortiz@yandex.ru *corresponding author

Aleksey A. Gryzunov – senior research scientist, Laboratory of refrigeration processing and storage of food products, All-Russian Scientific Research Institute of Refrigeration Industry – Branch of V. M. Gorbatov Federal Research Center for Food Systems of the RAS, 127422, Moscow, Kostyakova str., 12. Tel.: +7–495– 610-80-92, e-mail: grizu-nov@rambler.ru

Anna A. Vankova - candidate of biological sciences, docent, Department of microbiology and immunology, State Agrarian University - Moscow Timiryazev Agricultural Academy, 127550, Moscow, Timiryazevskaya str., 49. Tel.: +7-499-976-09-66, e-mail: anna.vankova@gmail.com

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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