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Comparison of the effect of ultraviolet light, ozone and heat treatment on muesli quality

I. Lignicka*, A. Balgalve, K. Ābelniece and A.M. Zīdere-Laizāne

Felici LLC, Rigas gatve 8, LV-2164, Adazi, Adazu novads, Latvia
*Correspondence: ilva.lignicka@musli.lv

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Abstract. Various muesli processing technologies can be used to lower microbiological parameters. It is necessary to find the best treatment technology to ensure that the product can meet various regulatory limits and to increase the shelf life of the product. The aim of this study was to compare the effect of ultraviolet light, ozone treatment and sterilisation on muesli quality. Muesli samples with dried fruits were tested, comparing the change in total plate count, yeast count and mould count. Short-wave ultraviolet (UV-C) light with a wavelength of 254 nm was used for ultraviolet light treatment, and the product was treated for 1, 2, and 5 minutes. As for ozone treatment, the samples were treated with an ozone concentration of 35 ppm for 30 minutes. Heat sterilisation was performed using sterilisation mode 25-30-50 (heating, holding, cooling), 119 °C, 2.2×10^5 Pa. Ultraviolet light and ozone treatment did not have a significant impact on total plate count, yeast count and mould count. Heat sterilisation had the most significant effect on muesli sample microorganism level, total plate count, yeast count and mould count level were 10 log cfu g^{-1}.

Key words: muesli, microbiology, heat treatment, ozone, UV.

INTRODUCTION

Muesli is a mixture of different raw materials such as cereal flakes, dried fruits, seeds, and nuts. Usually, all raw materials have different microbiological parameters, moreover not all of them are microbiologically safe. Globalization is increasing and products are shipped to different countries with different legislations and limits. In order to lower the microbiological parameters and extend the product shelf life, different muesli processing technologies can be used.

The wavelength of ultraviolet (UV) light ranges from 100 to 400 nm in the electromagnetic spectrum. UV radiation is divided into four segments, vacuum UV from 100 to 200 nm, short-wave ultraviolet (UV-C) from 200 to 280 nm, medium-wave (UV-B) from 280 to 320 nm, and long-wave UV (UV-A) from 320 to 400 nm. The highest germicidal efficacy is shown in the UV-C range (Umagiliyage & Choudhary, 2018). The basic principle is that UV at certain wavelengths destroys and damages the DNA of various types of microbes, rendering them inactive so that they cannot reproduce and multiply (Vasuja & Kumar, 2018). In the food industry, ultraviolet light is mostly used to disinfect equipment surfaces and decontaminate conveyor surfaces and

packaging containers. Despite the effectiveness of ultraviolet light for disinfecting smooth surfaces, there are relatively few applications of this technology in the food processing industry (Koutchma, 2008). The advantages of ultraviolet light treatment over other disinfection methods are obvious: no chemicals are used, it is a heat-free process, changes in colour, taste, odour, and pH are minimized (Sorour et al., 2014)

Ozone treatment is a chemical method of decontaminating food by exposing the food to ozone in aqueous and/or gaseous phase. The bactericidal effect of ozone has been confirmed for a wide range of microorganisms, including Gram-positive and Gram-negative, as well as bacterial spores (Brodowska et al., 2017). Ozone is the attractive choice for food processing and preservation industries to ensure microbial food safety because it is fast acting and has strong oxidative properties (Pandiselvam et al., 2017). This is the reason why the application of ozone both improves the microbiological safety of foods and extends their shelf life without significantly altering their nutritional, chemical, and physical properties. Ozone can be generated by the exposure of air or another gas mixture that contains oxygen to a source of energy such as high-energy electrical field (corona discharge method), ultraviolet radiation (phytochemical method), or conversion of oxygen molecules to ozone (chemical method). Mostly only two methods are used in practice: photochemical (UV) and corona discharge (CD). First one seems to be the most applicable in the food industry (Brodowska et al., 2017). CD discharge generators require an air preparation prior to ozone generation. The two common types of gas to feed CD ozone generators are oxygen and dry air. Product is treated in treatment chamber. It is collector where ozone enters from the top and returns though the bottom outlet after completing the exposure time. There are two electrodes in corona discharge, the high tension and lows-tension electrode, separated by a dielectric medium in a narrow discharge gap. When electrons have sufficient energy to dissociate the oxygen molecule, a certain fraction of these collisions occur, and a molecule of ozone can be formed from each oxygen atom. The outlet is connected to ozone analyser and then to ozone destructor. Ozone analyser is a device that is used for measuring the ozone concentration. At the outlet of the ozone destructor, excess ozone is destroyed, and the cleaned and decontaminated air is re-circulated to its intended enclosure or discharged to the ambient atmosphere (Prabha et al., 2015).

Sterilisation is the complete destruction or elimination of all viable organisms in/on an object to be sterilised. Sterilisation destroys yeasts, moulds, vegetative bacteria, and spore-forming organisms and allows food processors to store and distribute products at ambient temperatures, thereby extending shelf life. The sterilisation process includes four distinct stages. First, the product is heated to 110 to 125 °C to ensure sterilisation. Then, the product takes a few minutes to equilibrate as the surface is hotter and the middle part of the container is still cool. The equilibration phase allows the temperature gradient to be reduced. Then, the product must be kept at this temperature for a certain time to ensure a predetermined sterilisation value, which is determined by the F0 value. At the end, the product must be cooled, mainly to prevent further heat treatment and to avoid bursting of the container in hot conditions (Ramesh, 2003).

The microflora of cereals and cereal products is varied and includes moulds, yeasts, bacteria (psychotropic, mesophilic, and thermophilic), lactic acid bacteria, rope forming bacteria (*Bacillus* spp.), bacteria pathogens, coliforms, and Enterococci (Bullerman & Bianchini, 2008). During previous tests it is observed that in similar muesli samples no pathogen bacteria has been detected also coliform and Enterococci amount is

predictable. Most changing is total plate count, mould count and yeast count. There are basically no studies about different muesli treatments to reduce and control their microbial level. Most studies are concerned with one raw material treatment. Muesli is a mixture of different raw materials such as cereal flakes, dried fruits, seeds, and nuts. It is a non-homogenous product and usually, all raw materials have different microbiological parameters and irregular surface. The aim of this study was to compare the effects of ultraviolet light, ozone treatment and sterilisation on muesli quality. It is necessary to find the best treatment technology to ensure that the product can meet various regulatory limits and to increase the shelf life of the product. During this study, the change in total plate count, yeast count and mould count were compared. To compare the results obtained, European Union and Chinese legislation for microbiological limits were used.

MATERIALS AND METHODS

All muesli samples were prepared and supplied by Felici LLC. For this study, muesli samples were prepared with 79% cereal flakes and 21% dried fruits and vegetables. The recipe contained various ingredients such as dried date pieces, goji berries, carrot powder, mulberries, apple pieces, and raspberries. Muesli samples for each treatment experiment were prepared at different times. Before each experiment, the total plate count of the muesli, mould count, and yeast count were analysed. The total plate count was performed according to the method LVS EN ISO 4833-1:2014, while the mould and yeast counts were performed according to the method LVS ISO 21527-2:2008.

Ultraviolet light

Ultraviolet light Bactostop 30 lamp (Bactoscop, Poland), power 30 W, was added to the horizontal mixer. A total of 80 ± 0.1 kg of muesli sample was prepared using Matas (KEMEK Engineering, Lithuania) balances and added to the horizontal mixer. Short wavelength ultraviolet (UV-C) light with a wavelength of 254 nm was used. Muesli samples were mixed and irradiated with ultraviolet light during mixing (Fig. 1). The samples were mixed for one, two and five minutes. Ultraviolet light treatment was performed twice. After each treatment, muesli sample was taken to analyse the total plate count, mould count and yeast count. Muesli samples were mixed and treated according to the muesli production technological process to see if ultraviolet light treatment can be used during muesli production.

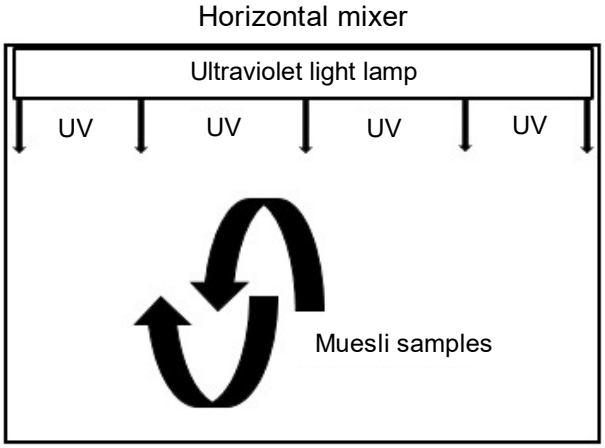

Figure 1. Designed scheme of ultraviolet light treatment.

Ozone treatment

Corona discharge ozone generator DNA-20G (Dino purification, China) was used for ozone treatment, capacity 20 g h^{-1}. Ozone was generated from ambient air. Ozone concentration was measured by ozone device QL -800-Q3 (QLOZONE, China). For ozone treatment, 100 ± 0.01 g of the sample was weighed (KERN & Sohn GmbH, Germany). Muesli samples were treated in the manufacturer laboratory, samples were treated in ozone concentration of 35 ppm for 30 minutes (Fig. 2) according to the manufacturer recommendation, based on previous tests for cereal flakes. After passing ozone through the product, the gas was directed to thermal ozone destructor. Clean and decontaminated air was discharged to the ambient atmosphere. Ozone treatment was performed twice.

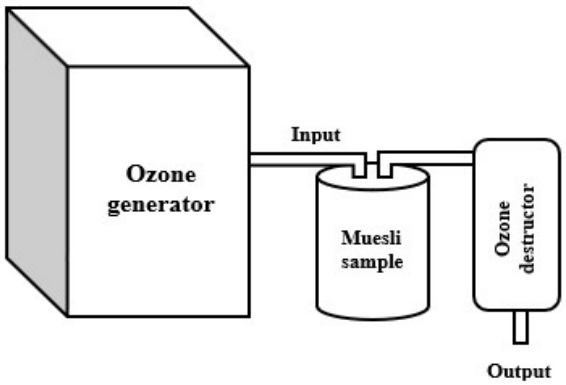

Figure 2. Designed scheme of ozone treatment.

Heat sterilisation

First, 100 ± 0.01 g of the sample was weighed (KERN &Sohn GmbH, Germany) and poured into a glass jar. The filled glass jars containing the muesli samples were sterilised using Steriflow device (Steriflow, France). Sterilisation mode 25-30-50 (heating, holding, cooling), 119 °C, 2.2×105 Pa (Fig. 3). The maximum temperature in the muesli sample during sterilisation was 112 °C. Sterilisation treatment was performed twice.

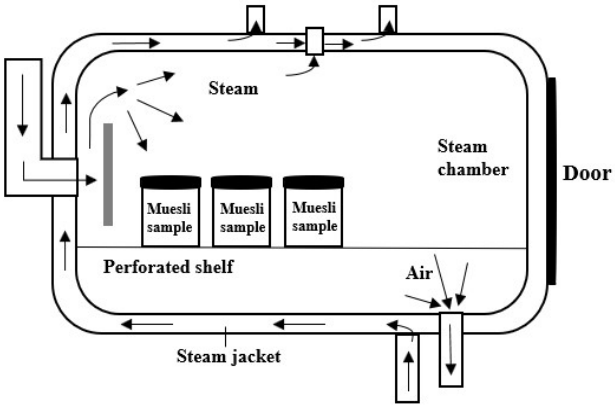

Figure 3. Designed scheme of sterilisation treatment.

Data processing

MS Excel 2016 was used to analyse the data obtained. ANOVA analysis was done to determine the difference between the samples. Samples were tested in triplicate. Factors were defined as significant if the p-value was less than 0.05.

RESULTS AND DISCUSSION

Table 1 shows the content of microorganisms in the commodities according to their specifications. It shows that the total microbial count varies from 10^4 to 10^5 cfu g^{-1}, while the mould and yeast count vary from 10^2 to 10^4 cfu g^{-1}. The highest microbiological counts are found in dried fruits and vegetable powders. As with cereals, microbial growth can occur during the wet phase, but further processing involves heat treatments that reduce the microbial load (ICMSF, 2005). Breakfast cereals processed in this manner under good sanitary conditions typically have aerobic plate counts of 1,000 cfu g^{-1}

(Deibel & Swanson, 2001). In a study of the microbial flora of whole grain cereal products in Germany, the aerobic plate count was about 10^6 cfu g^{-1}, but the fungal count was up to 2.0×10^5 cfu g^{-1}.

Table 1. Microorganism levels in different raw materials according to their specifications

Raw material	Total plate count, cfu g^{-1}	Mould count, cfu g^{-1}	Yeast count, cfu g^{-1}
Grain flakes	$\leq 1.0\times10^4$	$\leq 1.0\times10^2$	$\leq 1.0\times10^2$
Dried date pieces	$< 5.0\times10^4$	$< 1.0\times10^4$	$< 1.5\times10^3$
Dried goji berries	$< 1.0\times10^5$	$< 1.0\times10^4$	$< 1.0\times10^4$
Dried carrot powder	$\leq 1.0\times10^5$	$\leq 5.0\times10^2$	$\leq 5.0\times10^2$
Dried mulberries	$< 7.5\times10^4$	$< 1.0\times10^4$	$< 1.0\times10^4$
Dried apple pieces	$< 1.0\times10^4$	$< 1.0\times10^3$	$< 1.0\times10^3$
Freeze dried raspberry	$< 5.0\times10^4$	$< 1.0\times10^3$	$< 1.0\times10^3$

Although bacterial and mould counts were high, pathogen counts were low. Nevertheless, the mycoflora of cereal products can be very diverse. Microbial spoilage of breakfast cereals is rare because they have a low water activity of less than 0.50. The results of Kince et al. (2018) study on water activity varied from 0.108 to 0.494. Outbreaks of foodborne diseases associated with cereals are not common. As for spoilage, low water activity of the product prevents microbial growth (ICMSF, 2005) because water activity affects various biochemical reactions and microbial growth in food (Syamaladevi et al., 2016). Unsulphured fruits were used for this formulation. The microbiology involved in the processing of these fruits is described below. Mechanical dehydration reduces the overall microbial load, but the extent of reduction depends on both the type of fruit and the severity of the process. Poor sanitary conditions in the factory can lead to contamination of dried fruit during packing. Thus, the microbial population varies according to the type of fruit and the conditions of cultivation and processing (ICMSF, 2005).

Guirguis (2018) reported aerobic mesophilic bacteria in the range of 10^2 to 10^3 cfu g^{-1} and moulds and yeasts in the range of 10^3 to 10^4 cfu g^{-1}. For dried vegetables, the flora depends to a considerable extent on whether the flora of the raw, cleaned product has been largely destroyed by blanching. Vegetables for drying may promote microbial growth if kept at room temperature for too long. Populations ranging from 10^3 to 10^4 cfu g^{-1} have been reported on produce dried by blowing hot air through vegetables on perforated trays or belts (ICMSF, 2005).

Table 2. Calculated and analysed microorganism level in muesli samples

Muesli sample	Total plate count, cfu g^{-1}	Mould count, cfu g^{-1}	Yeast count, cfu g^{-1}
Calculated	1.0×10^5	1.0×10^3	1.0×10^3
UV light treatment	3.8×10^4	4.2×10^2	9.3×10^5
Ozone treatment	4.8×10^2	3.6×10^2	1.1×10^5
Sterilisation	6.5×10^2	9.1×10^1	1.7×10^4

Because the muesli samples were prepared at different times for each treatment experiment, muesli total plate count, mould count, and yeast count analyses were performed prior to each experiment. The calculated results and the results before each analysis are shown in Table 2. According to the written information in the raw material specification, the

possible levels of microorganisms in the muesli were calculated. The results are as follows - total plate count 1.0×10^5 cfu g^{-1}, mould count 1.0×10^3 cfu g^{-1}, yeast count 1.0×10^3 cfu g^{-1}.

The results show that the total plate count and mould count were lower than calculated and ranged from 10^1 to 10^4 cfu g^{-1}, while the yeast count was higher than calculated and ranged from 10^4 to 10^5 cfu g^{-1}. It can be seen that the microorganism content does not always meet the specification and may vary from batch to batch. No specific microbiological criteria for ready-to-eat breakfast cereals or muesli are mentioned in European Commission Regulation (EC) No. 2073/2005. Chinese legislation for microbiological limits was used for comparison. The national food safety standard GB 19640-2016 for cereal products is: total plate count 1.0×10^5 cfu g^{-1}, mould count 1.0×10^2 cfu g^{-1} and yeast count 1.0×10^2 cfu g^{-1}. Looking at the calculated and analysed microorganism content in the cereal sample, the total plate count complies with the regulations, but the mould and yeast counts exceed the limits. Nebrink (2007) reported that the allowable value of total plate count of 10^4 to 10^5 cfu g^{-1} is within the acceptable range. As for water activity, Semicenkova et al (2019) reported results of 0.36 for muesli with seeds, raisins, and chocolate. In another study, Senhofa et al. (2014) reported water activity of 0.56 for muesli with seeds.

The content of microorganisms in the cereal after each treatment is shown in Figs 4–6. The content of microorganisms in the muesli after sterilisation is shown in Fig. 4. The results show that the total microbial count, mould count and yeast count were 10 log cfu g^{-1} after sterilisation. The values show a significant difference ($p < 0.05$). Thermal food sterilisation and pasteurization are the most widely used preservation technologies to extend the shelf life of food by inactivating microorganisms and enzymes that can spoil food (Rodrigo et al., 2016). Food sterilisation is divided into two categories: Sterilisation by heating (thermal processing) and sterilisation without heating (non-thermal processing). Thermal processing is widely used nowadays, despite some problems, such as that the heating process could reduce the nutritional value or deteriorate the quality of the food, and that it is ineffective against certain types of bacteria. Non-thermal processing is considered an effective method that does not cause quality deterioration, unlike thermal processing. Nevertheless, there are no reports on the effect of sterilisation without heating (Ramesh, 2003). Some samples had changed their colour, which implies that different sterilisation methods should be tested to ensure low level of microorganisms and consistent quality.

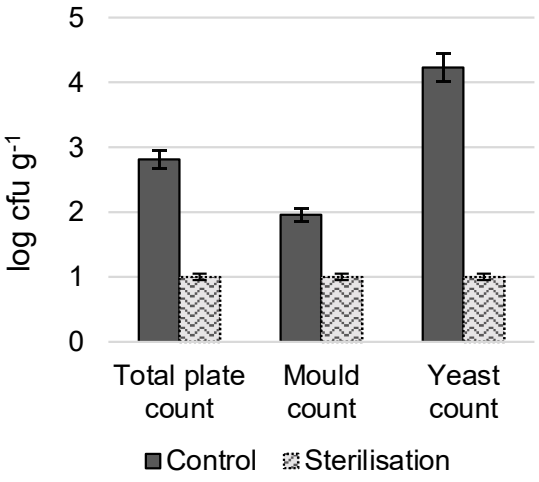

Figure 4. Microorganism level in muesli sample after sterilisation.

The content of microorganisms in the muesli sample after ozone treatment is shown in Fig. 5. The results show that the total microbial count decreased from 2.68 to 2.15 log cfu g^{-1}, mould count decreased from 2.56 to 10 log cfu g^{-1} and yeast count decreased from 5.04 to 4.54 log cfu g^{-1}. The values after ozone treatment showed a non-significant difference ($p > 0.05$). There are not many studies on the effect of ozone

treatment on the quality of muesli or breakfast cereals, mostly on the quality of cereal flakes or dried fruits. The study by Wu et al. (2006) showed that gaseous ozone for preservation of stored wheat was a very effective method to inactivate 96.9% of fungal spores associated with wheat. Higher treatment efficacy was achieved when the temperature and water activity of the wheat were increased. In another study, Dodd et al. (2011) reported on the effect of ozonation on malting barley that the treatment did not result in a significant reduction in aerobic plate count but did reduce mould and yeast counts by 1.5 log cfu g^{-1} in the finished malt. Gaseous ozone had no negative effect on any aspect of malt quality. Zorlugenc et al (2008) reported that the efficacy of gaseous ozone on aerobic mesophilic bacteria count (AMB) in figs was reduced by 0.81, 1.0 and 1.42 log cfu g^{-1} at 7.5, 15 and 30 min, respectively. Application at 7.5 min had a significant ($p < 0.05$) effect on the reduction, while application at 15 and 30 min was not significant. Total yeast count was reduced by 0.16, 1.57 and 2.09 log cfu g^{-1} at 7.5, 15 and 30 min, respectively. The reduction in yeast count was statistically significant ($p < 0.05$). Total mould count was reduced by 0.59 log cfu g^{-1} at 7.5 min. Moulds were completely inactivated at 15 min and 30 min. Najafi & Khodaparast (2009) reported the effectiveness of ozone in reducing microbial populations in date fruits. The study showed that the total number of mesophilic microorganisms was reduced from 4.06 to 3.80, 3.60 and 3.50 log cfu g^{-1}. For yeasts and moulds, the initial level was 3.93 log cfu g^{-1} and was reduced to 3.80, 3.63 and 3.50 log cfu g^{-1}. No visual changes for samples were detected.

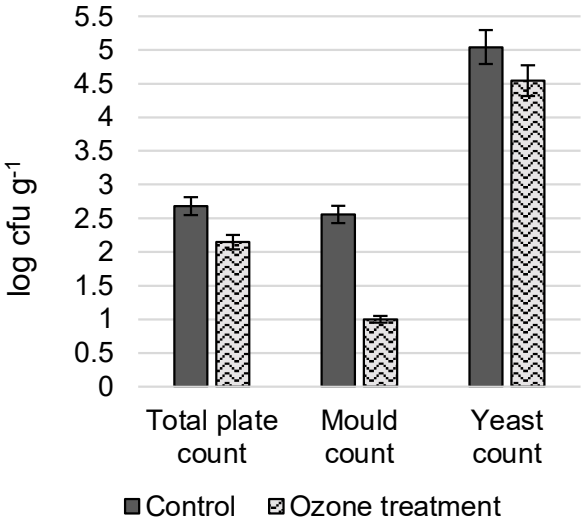

Figure 5. Microorganism level in muesli sample after ozone treatment.

The results after UV light treatment are shown in Fig. 6. It shows that the total bacterial count decreased from 4.58 to 3.89, 3.45 and and 3.38 log cfu g^{-1} after 1, 2 and 5 minutes of treatment, respectively. Mould count changed from 2.62 to 2.66, 2.08 and 10 log cfu g^{-1} after 1, 2 and 5 min of treatment, respectively. Yeast count decreased from 5.97 to 5.26, 5.14, and 5.99 log cfu g^{-1} after 1, 2, and 5 min of treatment, respectively. Pulsed UV light is considered more

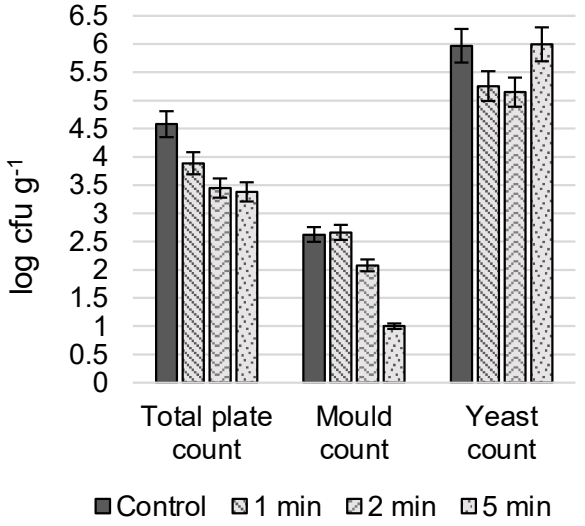

Figure 6. Microorganism level in muesli sample after UV light treatment.

efficient in microbial inactivation than continuous UV light and provides safer and faster decontamination. UV-C light, with the peak of maximum efficacy at wavelengths of approximately 260–265 nm, which corresponds to the peak of maximum DNA absorption, is most effective for inactivating microorganisms. The formation of cyclobutane-pyrimidine dimers during UV light treatment leads to mutagenesis and cell death. Although pulsed UV light is believed not to be a suitable technology for cereals due to the rough and uneven surfaces of cereals, the antimicrobial efficacy of this technology against microorganisms present on stored cereal grains has been demonstrated (Los et al., 2018). Maftei et al. (2013) study on decontamination of naturally occurring moulds on wheat grains achieved a reduction of about 4.0 log cfu g^{-1}, it also showed that the initial mould load of the grains is an important factor for treatment efficacy. No visual changes for samples were detected.

CONCLUSIONS

This study shows that microorganism level is variable, which means that treatment before packing would be preferable to be sure that results always meet the specification. Results show that UV light and ozone treatment had no significant impact on muesli sample microorganism level. Results show that the suggested UV light lamp cannot be used during the production process to decrease microorganism levels. This study also shows that the manufacturer suggested ozone concentration for cereal flakes cannot be applied for muesli treatment. All muesli raw materials have different microbiological parameters and irregular surfaces. The surface properties of food also influence ozone and ultraviolet light inactivation of microorganisms in dried foods. Heat sterilisation had the most significant effect on muesli sample microorganism level. Yet, it is needed to find the most appropriate sterilisation method. Some samples had changed colour what means that different sterilisation regimes or nonthermal methods should be tested to ensure low microorganism levels and equable quality. Nutritional and sensory properties need to be evaluated.

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