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Silver-clay polyethylene nanofilms effect on bread attribute

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ABSTRACT

In this study, the effects of seven nanofilm types on organoleptic properties have been investigated and microbial counts of packed volume bread have been studied. Packed of volume bread has been evaluated due to 21 days at three temperatures. Microbial counting was done in 5°C, 20°C and 35°C in fourteenth day. The results analysis indicated that the main factors such as film type, storage temperature and duration had significant effect on the bread staling rate. The lowest and highest staling rate was observed at SC3 film and control regular respectively. With the increasing nanosilver percentage, the total number of microbes declined. In three temperatures greatest number of total germs and mold was related to the control regular. Lowest and the most number of total molds and microbes was found in 5°C and 35°C respectively. Therefore, the SC3 hybrid nanofilm, has been recognized as the best film for keeping bread more fresh.

Introduction

Staling is the name of physico-chemical change which reduce quality and shelf life of bakery products. Staling is a term which indicates decreasing consumer acceptance of bakery product by changes in the crumb other than those resulting from action of spoilage organisms (Azizi, 2004a).

Bakery products packed, is mainly limited by two factors: atmospheric oxygen effect and the growth of aerobic spoiling microorganisms (Rodriguez et al, 2000). Staling refers to a broad set of sensory and chemical changes that affect the crispiness of the crust, the textural properties of the crumb and the aroma (Delnobile et al, 2003).

Some of the changes which occur in bread as a result of staling are: a) increase of crust and center of bread firmness, b) deterioration in flavor and reduce the moisture and freshness. Reducing of the bread softness during the time has been investigated by researcher (Shahedi, 2004).

bread staling rate and speed of getting stale measurement have several ways such as 1) chemical (total dissolved solids change, starch solution modification, Turgescence and humidity rate changes), 2)mechanical_ rheological methods (firmness and stiffness of bread, using Materials testing machine, using bakery density gauge, Farinogram profile change, amilogram profile change), 3)enzymatic methods, 4)electrical methods, 5) thermal methods [DSC and DTA] 6) organoleptic and sensory methods (kimiagar, 1996).

Spoilage usually occurred by mold, bacteria and viruses. Unusual metabolic changes because of too much heating, freezing, lack of oxygen or carbon dioxide stress, can cause tissue changes such as discoloration, notching, tainting, and too much softness texture changes (karim, 1997).

That damaged or spoilaged crop, with unacceptable sensory characteristics have not been accepted by consumers and spotted as wastage. Reasons of this kind of wastages are (Azizi, 2004b):

1)Microbial contamination and degradation

2)Metabolic processes

3)Physical stresses

fungi is a member of a large group of eukaryotic organisms such as yeast and mold .mold live as saprophyte and their spores can be found everywhere. Mold easily cause contaminate the foods. Mold can live and grow in different condition; 1) humid environments, 2) acidotic environments, 3) food containing large amounts of salt and sugar, 4) low temperature and also in presence of antibiotics. These microorganisms can intake Glusids, protein, fat and other organic materials which found in food and they cause color, smell and taste changes. Besides exterior spoilage, some fungi can produce dangerous toxins which cause mild, severe or lethal poisoning.

Presence and amount of fungi and mold in the food material from point of view of healthy food and trade is important and in health inspections, total count of mold is referred (karim, 1997). Wastage can be eliminated through processes, such as canning, freezing, drying, packaging etc. by using these methods, important changes may be occurred in the quality of the product. We can decrease wastage to minimal amount in different Packaging level such as; bulk packaging, packaging in consumer level, either at the production line or in the processing place, or primary, secondary or tertiary packaging stages (afshari & Minaee, 2008). Nano composites or nano-films can increase heat-resistance properties and mechanical strength, and reduce the oxygen transfer rate (Sattari Najaf Abadi et al, 2010).

Lines et al (2008), counted the number of microbes inside the stored bread at temperature 5° C during 28 Days. The results also were the same as Lenushner and colleagues (1997) labrotary test which showed, microbial activity starts after the fifteenth day. They believed by using the refrigerator; microbial growth will be prevented. In another study, Karaughlu et al (2005) examined microbial properties of bread at a temperature of 20°C for seven days. They demonstrated that the growth of microbes in refrigerator has been reducing greatly.

One factor that may affect the staling process is the difference in water content and mobility between two breads (Fernandez et al, 2006). Although firming of bread crumb is known not to be due to the loss of moisture, it is a fact that the

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firming rates as well as the rate of starch recrystallization are sensitive to the actual water content during (Czuchajowska et al, 1989; Eliasson & Larsson, 1993).

Rate of firming has an inverse relationship to crumb moisture content (Rogers et al, 1988). Moisture migrated during storage from crumb to, and within bread constituents (Avital et al, 1990; Morad et al, 1980; Kelp et al, 1981). Piazza and masi (1995) reported a correlation between local moisture content and texture of stale bread. Platt et al (1940) found a strong correlation between the staling rate of bread and moisture content. Bechtel & Meisner (1954) concluded that bread with higher moisture content was significantly fresher than bread with a lower content. Bashford and Hartung (1976) found good correlation between sensory and Instron measures of bread.

The nanofilm includes the nanosilver and clay. Nanosilver content in the film give the antibacterial properties and the clay will prevent oxygen and water vapor permeability. Silver nano-particles with antimicrobial properties prevent the growth of mold and microbes and provide the anti microbial environment which lead to improve Organoleptic properties of bread. Applying the clay nano-particles increased the penetration path across the film polymer. Therefore Reduces water vapor permeability through the packaged of bread which helps to keep the bread fresh (Sattari Najaf Abadi et al, 2010). In different maintenance conditions the interaction effect between storage time, temperature and type of film on volume bread has been investigated. In this study, organoleptic methods (sensory) were selected to evaluate effects of nano silver and nano clay on Staling and appearance of bread simultaneously.

Materials and methods

Nano-liquid interaction based on polyethylene method was used for making seven nanofilms types. The thicknesses of these films were less than 100 microns. Breads acquired from Nan-Avaran Company and packed into the nano film and packed by an electric sewing machine.

Volume Bread packed into nano films were evaluated in day 1, 3, 5, 7, 14 and 21 (storage period) at temperatures 5, 20 and 35 Celsius degrees. At the day of fourteenth Microbial samples counted at temperatures of 5, 20 and 35 Celsius degrees.

Control film (no treatment film) is ordinary nylon (LDPE) (Low Density Poly Ethylene). Nanosilver particles and nano-clay added to the films during melted interaction method. The Range of density for nanosilver particles is between 250 ppm up to 1000 ppm. Values less than 250 ppm, has little anti microbial effect and values more than 1000 ppm, is not economic. The Range of density for nano-clay particles is between 150 ppm up to 450 ppm. Using the 150ppm and less than this amount is not effective while use 450ppm and more than this amount has negative effect on film color. Changes of the color in packages are very important case in food products (Sattari Najaf Abadi et al, 2010).

Bread staling test

73-74AACC method was used for evaluate Bread staling (AACC, 1990). In 1954, Bechtel and Meisner educated a group of people to study bread changes according to the following principles:

- Touch a piece of bread by Finger to detect stiffness and softness. Obviously stale bread is more rigid.
- During staling process fresh taste of bread is lost and bad taste emerges.
- Fresh bread taste moist and has sticky center but the center of staled bread is dry and fragile.

The organoleptic test was done. Samples of bread was sliced, 10 judges and 2 experts evaluated the samples in days 1, 3, 5, 7, 14, 21 (in the room temperature). Samples were scored. Forms which used for evaluation is showed in tables 2.

Microbial Test

Microbial counting methods include standard techniques of total counting, mold counting and coliform counting (AOAC, 1984). The total count and mold count has important role in this experiment so this counting was done at the fourteenth day. Counting bacterial colonies on agar plates is an effective method for determining the number of viable bacteria in samples. This method relies on the growth of a bacterial cell in an agar plate to form a visible colony, only living or viable bacterial cells will be counted. Aseptic technique is used for all steps all plates, Pipettes and medium autoclaved. There are three major steps in this test: preparation of serial dilutions, mixing the serial dilutions into agar, counting the resulting bacterial colonies

Preparation of Serial Dilutions

10gr of samples was taken in to the peptone water then the samples mixed well to provide liquid sample with 0.1 dense. 9 mL of isotonic diluent were Pipetted into each bottle. 1mL of the undiluted sample Pipetted into the bottle marked 10^{-1}. The contents mixed and 1 mL from the 10-1 bottle pipetted into the 10^{-2} bottle. This procedure was continued until transfers have been completed to the 10^{-3} bottle.

Mixing the dilutions into agar plates

Autoclaving of potato dextrose agar (PDA) was done in 121°C. The bottle of molten agar Placed in a 50°C water bath and allow the agar to cool to 45°C.about 15 mL of molten agar poured into each plates. Each plate swirled gently to mix the 1 mL of diluted samples into the 15 mL of agar. The plate Leaved without moving for at least 13 minutes to allow the agar to set. When the agar was set, the plate incubated at 35°C for 48 – 72 houres. After three days the colony counting was done in three repeat on plate count agar (PCA). For total counting the $10^{-1,}$ 10^{-2} density was used for 5 Celsius degree and the 10^{-2}, 10^{-3}density was used for 20 and 35 Celsius degree. For mold counting the 10^{-1}, 10^{-2} density was used for 5 Celsius degree and the 10^{-2}, 10^{-3} density was used for 20 and 35 Celsius degree.

Statistical analysis

At the end, all test results analyzed with SPSS (version 14) software, (ANOVA) test at $\alpha = 5\%$. If ANOVA results were significant, a Duncan multiple range comparison tests were done.

Results and discussion

The effect of various factors such as type of film, storage temperature and maintaining duration on organoleptic and microbial characteristics are provided.

Bread Staling test by taste

As seen in Table 3, the effect of three main factors such as types of films, temperature and storage time on the bread staling scores, was significant. Dual interaction at two statistical levels 1% and 5% was significant. But the triple effect of "film type × temperature × storage period" was not significant statistically. Signification of dual effect impressed main effects meaningful. So, dual interactions are checked.

Various types of nano-films Effect on quality and bread staling characteristics in three temperatures

Effect of the type of Film on bread staling scores, at three temperatures 5, 20 and 35 Celsius degrees are showed in fig. 1.

With increasing storage time, bread Staling scores will be decrease (lower score = increased Staling). But this decreasing trend at various temperatures is different. According to Fig. 1.A, at the 5°C, bread has better shelf life than the temperature 20° C and 35°. The point is in the third day, suddenly the bread staling score decrease. This procedure will become very slow at the end of the twenty first day of experiment. The score is higher at temperatures 5°C because of delays in the mold spots emergence. At the temperatures of 20 °C and 35°C, bread get moldy earlier. At both temperatures 20 °C and 35°C, bread Staling rate score decreased slowly until seventh days, which after that day dramatic decrease was observed. In the fourteenth day a lot of differences were observed between the scores of different kind of films at these three temperatures. On the Twenty-first day, none of the packages were usable and got Staling score of 1 (lowest score). Finally, significant differences between nano-films scores and control samples were observed. So that bread staling scores in nano film packages were 20% to 40% percent is higher than the control treatment (20% to 40% less Staled). According to the fig. 1, after fourteen days the lowest Staling score (highest Staling scores), occurred in hybrid nano-film SC3 and highest staling score related to the control regular (W).

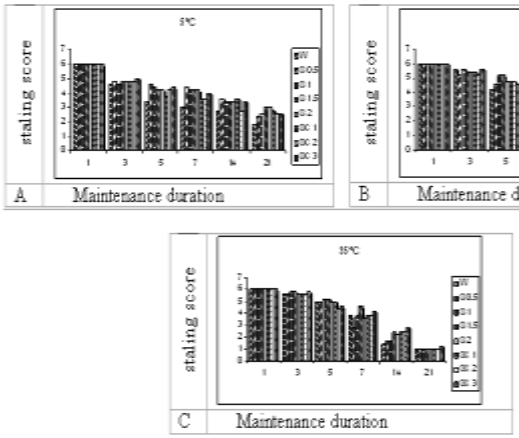

Figure 1 The effect of films type on bread staling score during keeping (A: 5°C, B: 20°C, C: 35°C)

Silver has anti-microbial properties and prevents microbial growth. Increasing the percentage of silver in various films type can cause anti bacterial environment in package, therefore lower spoilage level was observed in control sample. Silver particles prevent growth of mold and cause better quality and appearance of packed bread. Clay prevents outgoing of water vapor and oxygen from package of bread, therefore moisture remains inside the package and start of staling inside the bread was delayed. So with increasing the amount of clay in hybrid silver - clay films the Staling rate decreased. It should be mentioned that one of the reasons for bread staling is the loss of moisture. Nano-films by preventing moisture cause increasing of bread shelf life.

Effect of temperature on the characteristics of qualitative and bread staling in different type of films

Fig. 2, showed the effect of temperature and storage time in a different type of films. Comparison of test results is showed in figure below. Column with the same letters do not have significant difference at 0.05 levels.

In Fig. 2, interaction of temperature and storage time in a various films showed. It should be notice that the columns with the same letter don't have significant difference at statistical level of 5%. As shown in Figure 4 the control sample (W) at temperature 5 ° C, sudden decrease in bread staling rate is observed, after the seventh day the speed of staling decreased. At 20 C ° and 35C °, until the fifth day staling score's loss has little reductions. But after the fifth day the sudden Staling reduction scores were observed. This indicate that in 20° C and 35 ° C, much initial refreshness keep in bread than 5° C, but due to the rapid loss of moisture, the bread become staled sooner and after a while mold appeared. However, at the temperature 5°C, the bread staling rate is faster but mold appeared with delay. So the better shelf life was observed at temperature 5°C.

Figures presented percentage and composition of nano films separately. Differences in Staling score of various types of nano films represented. Increasing trend of Staling is the same in all films. For example, at the temperature 35°C, the sudden loss of Staling score in most nano films occurs on the seventh day but in the case of control sample, the decrease observed on the fifth day, This indicates that the ability of nano-films in maintaining freshness of bread. As it shown in Table 3, the effect of temperature was significant at the level of 5%. That was opposite of our hypothesis which stated the non significant effect of temperature on nano-film. This happened Because of the differences between nano-film manufacturing methods of and film quality which made in Iran and the other countries. Total results showed that the nano film has positive effect on packaged breads quality and bread shelf life.

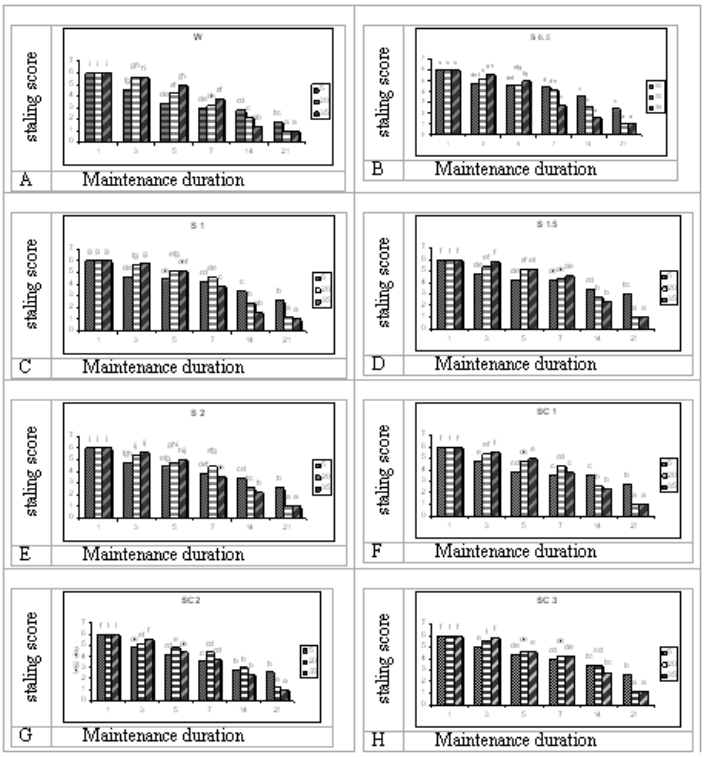

Figure 2 shows effect of temperature and time on the amount of bread staling in a different type of films during 21 days (A: W, B: S 0.5, C: S 1, D: S 1.5, E: S 2, F: SC1, G: SC2, H: SC3)

Effect of temperature and film types on the microbial characteristics of packed volume bread

Total counting and mold counting

As it is seen in the tables (4 & 5), the main effect and interaction effect between temperature and film type on the total count and mold counts was significant at 1% level.

Effect of various nano films on total microbial counts and molds in three temperatures

Effect of nano-films on total microbial counts and molds at 5°C, 20°C and 35°C were shown in fig. 3. As it was expected, influence of silver nano-particles was obviouse. In compared with the control sample With increasing the percentage of nano-silver , mold count decreased dramatically, but in the nano-film with silver particle (SC2), the amount of total microbial and molds counts increased this was because of testing error or film quality.

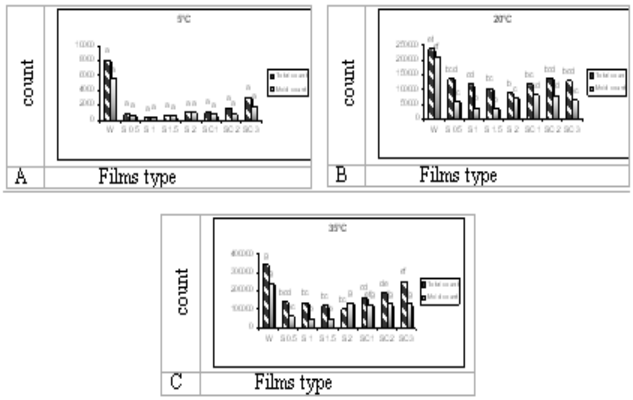

Figure 3 Effect of kind of nano-films type on Total microbial count and mold count at three temperatures 5°C, 20 °C and 35°C (A: 5°C, B: 20°C, C: 35°C)

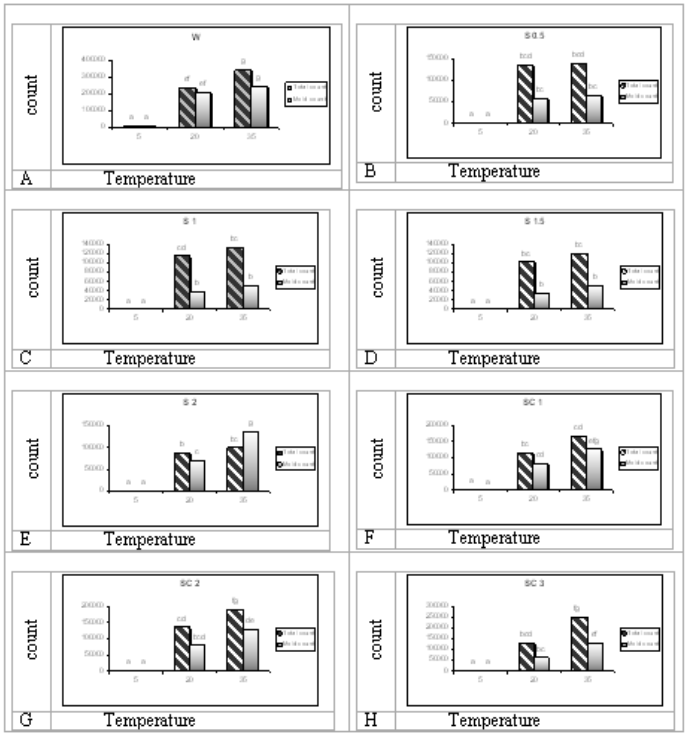

Figure 4 Effect of temperature on the total number of germs and mold in different types of films (A: W, B: S 0.5, C: S 1, D: S 1.5, E: S 2, F: SC1, G: SC2, H: SC3)

In the case of total microbial counts, with increasing percentage of nano-silver, the total number decreased. In the case of hybrid nano-films, contrary to expectations, with increasing clay percentage, the total number of germs and mold has been raised. This represents a negative effect of high clay particles density on the total number of microbes and mold. The reason is; with reduced water vapor permeability, appropriate conditions have been provided the growth of microbes and mold. Lowest total number of germs and molds in three temperatures was observed in the SC1 hybrid nano- films. In this film because of 1% nano-silver amount, has the lowest number of germs and mold than the control sample. Highest total count of germs and mold in three temperatures was found in control film (without nano-particles).

Effect of temperature on total microbial and mold counts

Effect of storage temperature on total microbial and mold counts of bread is shown in the figure 4. As it can be seen, with increasing temperature, a sudden increase in the total count of microbes and mold observed. 20°C and 35°C provide appropriate condition for mold growth. But 5°C temperature prevents growth of germs and mold.

As it can be observed with increasing temperature, the total number of germs and mold also increased. Therefore the lowest total number of germs and mold was found at the temperature of 5°C and the greatest number has been found at the temperature of 35°C.

Conclusions

- Nano silver and nano clay particles have significant effect on improve microbial and organoleptic properties of bread. Nano-Silver particles with their antimicrobial properties prevent mold and microbes growth and with providing the anti-microbial environment, this cause to improved Organoleptic properties of bread. Applying the clay nano-particles with a nanometric thin flat layers and can cause severity in penetrating the molecules through nano-composite mass. The presence of these particles increases the penetrating path through the polymer, so reduced outgoing of water vapor in the packages will help to keep freshness of breads and help to increase bread shelf life.
- Three main factors such as film types, temperature and storage time has significant Effects on the bread staling scores. Also, the dual interaction was statistically significant at two levels 1 and 5 percent. But triple effect of film type's × temperature × storage period on bread staling scres was not statistically significant.
- lowest Staling was observed after fourteen days keeping, in hybrid nano-films (SC3) and the Most Staling rate is related to the control film. Increasing the percentage of silver in the film can cause increase anti-bacterial properties of the product, so in compare with control sample total score was increased. In hybrid silver-clay films, with increasing clay percentage, the Staling rate was increased.
- At the two Temperatures 20°C, and 35 °C, initial freshness of bread preserved more than 5°C, but at the end because of delays on the emergence of spots mold, shelf life of 5°C is longer than the other temperatures.
- the interaction between film types and temperature has statistically significant Effects on total count and microbial count at 1% levels. Meanwhile The main effects has been statistically significant at 1% level.
- With increasing the density percentage of nano-silver up to 750Ppm, mold count decreased dramatically. The lowest microbial counts were related to nano-film with 1000Ppm silver density. The highest total germs and mold counts was related to control sample.
- With increasing temperature, the total number of germs and mold also increased. So that the lowest total number of germs and mold at three temperatures was related to the temperature 5°C, and the highest amount was observed at the temperature 35 °C.
- Finally, in the SC3 hybrid nano-film with 450 ppm silver -500 ppm clay, the greatest bread staling delay time was observed and nano film with silver (500 ppm silver) was the best film in terms of reducing the total number of germs and mold.

- Despite the production cost of silver nano-particles, is 1.1 fold to 1.3 fold expensive more than ordinary films and the cost of nano-hybrid films, 1.1 fold to 1.2 fold expensive more than ordinary films, using nano-films is more economic. For example as observed, in the case of nano-films, time of being staled increased 40 to a 60 percent, or by applying silver nano-films, the total number of germs and mold decreased significantly (Sattari Najaf Abadi et al, 2010).

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Table 1. The Principles for Nomenclature of films which used in this study treatment number treatment symbol Composition and percentage

Number of treatment	Treatment symbol	Composition and percentage
1	W	Regular Control
2	S 0.5	250 Ppm silver
3	S 1	500 Ppm silver
4	S 1.5	750 Ppm silver
5	S 2	1000 Ppm silver
6	SC 1	150 Ppm clay - 500 Ppm silver
7	SC 2	300 Ppm clay- 500 Ppm silver
8	SC 3	450 Ppm clay - 500 Ppm silver

Table 2. evaluation form for bread quality during 21 days of shelf life

Level	Score	Day					
		1	3	5	7	14	21
Very fresh	6						
Fresh	5						
Just fresh	4						
Just stale	3						
Stale	2						
Very stale	1						

Table 3. variance analysis of staling data

independent variable	degree of freedom	mean square	F
Films type	7	1.191**	5.875
temperature	2	0.817*	4.031
Maintaining duration	5	217.071**	1070.740
temperature× Film type	14	0.404*	1.955
Maintaining duration× Film type	35	0.322*	1.586
temperature× Maintaining duration	10	7.914**	39.039
temperature× Shelf life× Film type	70	0.265ns	1.305
error	342	0.203	
total	485		

ns: no significant ,* : significant at (5%) level And **: significant at (1%) level

Table 4. Total counts variance Analysis

independent variable	degree of freedom	mean square	F
Film type	7	1675453118**	12.692
temperature	2	242491241772**	182.700
Film type× temperature	14	5448945346**	4.128
error	48	1320038695	
total	71		

**: significant at (1%) level

Table 5. Variance analysis of mold counting data

independent variable	degree of freedom	mean square	F
Film type	7	3489877053**	16.085
temperature	2	63606226605**	293.165
temperature× Film type	14	1218353481**	5.616
error	48	216962036	
total	71		