Yüzüncü Yıl Üniversitesi
Tarım Bilimleri Dergisi
(YYU Journal of Agricultural Science)

http://dergipark.gov.tr/yyutbd

Araştırma Makalesi (Research Article)

Comparative Assessment of Potassium Sorbate and Clove Oil (*Syzygium aromaticum*, L.) on Quality Alteration in Chilled Crayfish (*Astacus leptodactylus*)

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Article Info

Recieved: 22.01.2020
Accepted: 25.03.2020
Online Published: 31.03.2020
DOI:10.29133/yyutbd.678889

Keywords
Crayfish,
Potassium sorbate,
Clove oil,
Quality,
Chilling

Abstract: In this study, comparative assessment of potassium sorbate and clove oil (*Syzygium aromaticum* L.) on the quality characteristics of crayfish (*Astacus leptodactylus*) during storage at 2°C for 20 days has been investigated. Both potassium sorbate and clove oil application retarded lipid oxidation during 20 days of storage. There were no significant differences between peroxide values (PV) of potassium sorbate and clove oil treated crayfish samples. At day 20, CO1 group showed the lowest FFA content. Least TMAB was observed in 1% clove oil treated samples throughout the storage period. Clove oil treated samples had significantly lower PBC then the control and potassium sorbate treated crayfish samples during storage period. After 10 days of storage, control samples were microbiologically unacceptable. 1% clove oil treatment was more effective than 1% potassium sorbate treatment in preventing growth of molds and yeast throughout the storage period.

Potasyum Sorbat ve Karanfil Yağının (*Syzygium aromaticum*, L.) Soğutulmuş Kerevitlerde (*Astacus leptodactylus*) Kalite Değişimi Üzerine Karşılaştırmalı Değerlendirmesi

Makale Bilgileri

Geliş: 22.01.2020
Kabul: 25.03.2020
Online Yayınlanma: 31.03.2020
DOI:10.29133/yyutbd.678889

Anahtar Kelimeler
Kerevit,
Potasyum sorbat,
Karanfil yağı,
Kalite,
Soğutma

Öz: Bu çalışmada, potasyum sorbat ve karanfil yağının (*Syzygium aromaticum* L.) 2°C'de 20 gün depolama süresince kerevitlerin (*Astacus leptodactylus*) kalite özellikleri üzerine karşılaştırmalı olarak değerlendirilmesi yapılmıştır. Potasyum sorbat ve karanfil yağı uygulaması, 20 günlük depolama süresince lipit oksidasyonunu geciktirmiştir. Potasyum sorbat ve karanfil yağı ile muamele edilmiş kerevit örneklerinin peroksit değerleri (PV) arasında belirgin bir farklılık bulunmamıştır. 20. günde, CO1 grubu en düşük serbest yağ asidi (FFA) değerini göstermiştir. Depolama süresi boyunca en düşük toplam aerobik mezofilik bakteri sayısı (TAMB) %1 karanfil yağı ile muamele edilmiş örneklerde gözlenmiştir. Depolama süresi boyunca karanfil yağı ile muamele edilmiş örnekler, kontrol ve potasyum sorbat ile muamele edilmiş kerevit örneklerine oranla belirgin olarak daha düşük psikrofilik bakteri sayısına (PBC) sahiptir. 10 günlük depolama sonrası kontrol numuneleri mikrobiyolojik olarak tüketilebilir özelliğini kaybetmiştir. %1 karanfil yağı muamelesi, depolama süresi boyunca küf ve maya gelişiminin önlenmesinde % 1 potasyum sorbat muamelesine oranla daha etkili olmuştur.

1. Introduction

While good quality fresh fish and seafood demand increases day by day, keeping the quality of seafood becomes an issue due to intensive transportation and distribution among nations. Fresh fish and seafood products are susceptible to deterioration from postmortem microbial growth and enzymatic activity (Manju et al., 2007). Since the controlling of natural enzyme in fresh fish is difficult, the measures taken are mostly controlling of microbial activity (Yetim, 1996).

Chilling is the most common method to retard the microbial and biochemical spoilage of fresh fish and seafood during distribution and marketing but it is not enough to maintain the quality. At low temperature, growth of bacteria is retarded but never completely stopped and this technique cannot prevent enzyme activities (Sampels, 2015). Therefore, chilling process should be combined with other preservation techniques to delay the microbial spoilage and to improve the quality of seafood.

Chemical preservatives are used commonly in food processing sector to increase the shelf life and to preserve the food. Potassium sorbate is one of the safest chemical preservatives that is generally used in fish and seafood products (Omojowo et al., 2009b). This food additive is a white crystalline powder, tasteless, odorless. It is the inorganic salt of sorbic acid with strong antimicrobial activity against bacteria, yeast, molds and fungi (Remisha et al., 2016). They are used alone or in combination with other preservation techniques (Nunez and Aquino, 2012).

Essential oils are natural preservatives and they are better and safer alternatives to chemical preservatives due to their non-toxic nature (Anand and Sati, 2013). Essential oils are aromatic oily liquids obtained from flowers, buds, bark, leaves, seeds, peel, fruits and herbs (Hyldgaard et al., 2012; Dabija et al., 2019). They are used in many areas such as cosmetics, aromatherapy pharmaceuticals, food and drinks (Arrijani et al., 2017). They are cheap and small quantity is enough to prevent the growth of microorganisms. Essential oils can be applied directly to the food or in emulsion and nanoemulsion forms (Ceylan et al., 2018; Fernandez-Lopez and Viuda-Martos, 2018: Meral et al., 2019).

Clove essential oil (*Syzygium aromaticum* L**.)** is obtained from dry flower buds of clove and it has antimicrobial, antioxidant, antifungal, antiviral, anti-inflammatory and cytotoxic properties due to the presence of eugenol, thymol and cinnamaldehyde (Chaieb et al., 2007).

Although there have been many publications on the effects of essential oils and potassium sorbate on fish quality, no studies have been conducted to compare them with each other. Therefore, the purpose of this paper is to compare the antioxidant and antibacterial activity of potassium sorbate and clove oil on quality characteristics of crayfish stored at 2°C for 20 days.

2. Materials and Methods

2.1. Materials

Crayfish (*Astacus leptodactylus*) were obtained from Keban Dam Lake. They were transferred to the laboratory in the styrofoam boxes containing ice and were processed in the same day. In this study, 7 kg of crayfish with an average weight and length of 70.56 ± 15.21 g and 16.03 ± 0.34 cm, respectively was used.

Clove oil was purchased from Kalsec (Kalsec®, Inc, Kalamazoo) and potassium sorbate (food grade, molecular weight: 150.22, CAS Number: 24634-61-5, purum p.a. ≥ 99%), was purchased from Sigma Aldrich Chemical Co. (Munich, Germany).

2.2. Experimental design

Crayfish were washed with cold clean water (2°C) to remove dirt and boiled for 10 minutes at 100°C. After boiling, the excess water was removed by spreading them on perforated trays and they were cooled to 4°C then they were separated from their shells. After separating, they were randomly chosen and divided into three lots. The first lot was kept as control (without dip treatment), 2nd lot (PS1) was subjected to dip treatment with 1% (v/w-) potassium sorbate solution for 3 minutes and 3rd lot (CO1) was subjected to dip treatment with 1% (v/w) clove oil solution for 3 minutes. When drainage is completed, all lots were placed in different styrofoam boxes, wrapped with stretch film and preserved at 2°C for 20 days.

All samples were analyzed for chemical (thiobarbituric acid (TBA), peroxide value (PV), free fatty acid (FFA)) and microbiological characteristics (total aerobic mesophilic bacteria (TAMB), psychrophilic bacteria count (PBC) and yeast-mold count) for every 5 days of interval.

2.3. Chemical analysis

TBA value was determined by the distillation method of Tarladgis et al., 1960 and expressed as mg Malonaldehyde/kg of crayfish sample. Peroxide value was calculated and expressed as milli-equivalent of O_2/kg fat and it was determined according to the method of AOCS, 1989. Free fatty acids (FFA) content was determined as described by Yetim, 2002 and calculated as oleic acid %.

2.4. Microbiological analysis

10 g of crayfish sample was mixed with 90 ml of sterilized distilled water. Further decimal serial dilutions (from 10^{-1} to 10^{-9}) were used from this homogenate. Total aerobic mesophilic bacteria (TAMB) and total psychrophilic bacteria counts (PBC) were enumerated on plate count agar (PCA) and incubated at 30°C for 3 days and 7°C for 10 days, respectively (ICMSF, 1986). Yeast and mold count was determined by yeast extract glucose chloromphenicol (YGC) agar with the incubation at 22°C for 5 days (Harrigan and McCance, 1976).

2.5. Statistical analysis

Statistical analysis was carried out using SPSS 22.0 (SPSS 22 for Windows, SPSS Inc. Chicago, IL, USA). All data were expressed in mean± SD. Variance analysis (ANOVA) was performed and means were compared by Duncan's multiple range test. The level of significance was set at $p<0.05$.

3. Results and Discussion

3.1. Chemical analysis

3.1.1. Thiobarbituric acid (TBA) value

TBA values of potassium sorbate and clove oil treated samples are shown in Figure 1. Initial TBA values of control, 1% potassium sorbate added (PS1) and 1% clove oil added samples (CO1) were 0.59, 0.56 and 0.54 mg MDA/kg of sample, respectively. From day 0 to 5, there was no significant difference in TBA values of control (1.24 mg MDA/kg of sample) and PS1 group (1.28 mg MDA/kg of sample). Genccelep et al., 2014 reported that during 6 days of refrigerated storage, no significant differences were observed between the control and potassium sorbate treated samples. Significant difference was observed in TBA values of CO1 group at day 0 and 5th. Clove oil was very effective in retarding lipid oxidation during 5 days of cold storage ($p<0.05$).

From day 10 to end of the storage, potassium sorbate application had significant effect on TBA values of crayfish samples when compared to control and clove oil treated samples ($p<0.05$). Potassium sorbate application was very effective between days 10 and 20. TBA values were low in potassium sorbate treated samples due to inhibition of secondary oxidation products by potassium sorbate (Gandotra et al., 2014).

The results are in agreement with Gandotra et al., 2014 and Remisha et al., 2016. Gandotra et al., 2014 found that potassium sorbate treated silver carp fillets showed lower values for TBA during 30 days of storage. Remisha et al., 2016 investigated the effect of potassium sorbate on the quality of Indian mackerel during 23 days of chill storage and they find that TBA values of control samples were higher than potassium sorbate treated samples and TBA values of potassium sorbate treated fish samples were below the acceptable limit value.

At the end of the storage period, TBA values of control, PS1 and CO1 groups increased to 5.12 mg MDA/kg of sample, 4.12 mg MDA/kg of sample and 4.61 mg MDA/kg of sample, respectively. While the control group exceeded the acceptable limit value between 5 mg MDA/kg of sample

(Dandago et al., 2004) at day 20, PS1 and CO1 were still below the limit value. Both potassium sorbate and clove oil application retarded lipid oxidation during 20 days of storage.

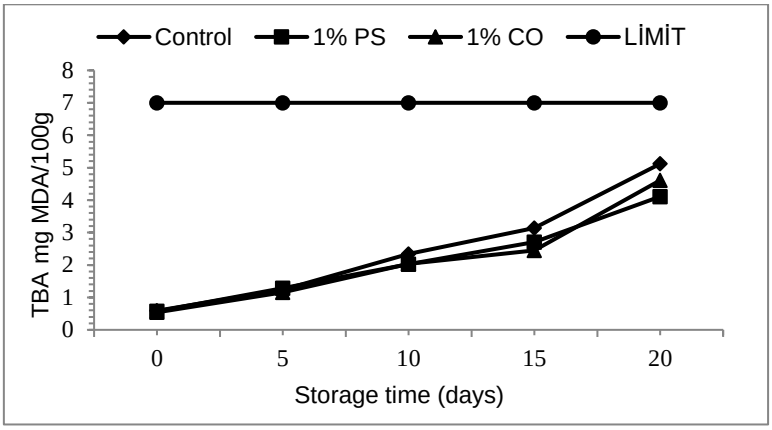

Figure 1. Changes in TBA values of potassium sorbate and clove oil treated crayfish samples during storage at 2°C. C control, PS1 1% (v/w) potassium sorbate, CO1 1% (v/w) clove oil.

3.1.2. Peroxide value (PV)

Peroxide value of crayfish samples treated with potassium sorbate and clove oil are shown in Figure 2. Initial peroxide values were 1.05, 0.93 and 0.91 meq O_2/kg for control, potassium sorbate and clove oil treated samples, respectively. As it seen, peroxide values of control samples increased sharply during storage period ($p<0.05$). Peroxide values in both potassium sorbate and clove oil treated crayfish samples showed slightly increase throughout the storage period and there was no significant difference between these samples ($p>0.05$). At the last stage of period, the peroxide values increased to 14.07, 7.30 and 7.19 meq O_2/kg for control, potassium sorbate and clove oil treated samples, respectively. It was clear that both potassium sorbate and clove oil showed antioxidant activity and they were very effective on lipid oxidation. The antioxidant activity of clove oil comes from its scavenging of free radicals and metal chelating ability (Chaieb et al., 2007). The antioxidant activity of potassium sorbate comes from its influence on the electroreduction of oxygen and its interaction with reactive oxygen species (Korotkova et al., 2006). Coban and Patir, 2013 found that lipid oxidation was higher in control samples than clove oil treated samples. Remisha et al., 2016 investigated the influence of potassium sorbate dip treatment on the shelf life of Indian mackerel (*Rastrelliger kanagurta*) during chill storage and they found that peroxide values of potassium sorbate dip treated samples were below the limit value of 10 meq O_2/kg (Varlık et al., 1993) during 18 days of chill storage.

Wu et al., 2019 reported that 0.2% potassium sorbate added chitosan coating significantly inhibited the increase in peroxide value of scallop during 8 days of cold storage.

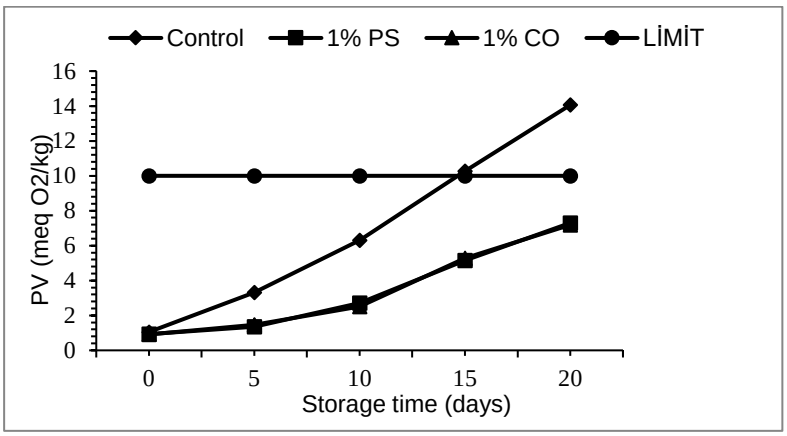

Figure 2. Changes in peroxide value of crayfish samples treated with potassium sorbate and clove oil during storage at 2°C. C control, PS1 1% (v/w) potassium sorbate, CO1 1% (v/w) clove oil.

3.1.3. Free fatty acid (FFA)

Lipolysis of triglycerides and phospholipids leads to formation of free fatty acids (FFA) (Pearson et al., 1983). Increasing of FFA content accelerates the oxidation of foods. FFA content of crayfish samples treated with potassium sorbate and clove oil are seen in Figure 3. At the beginning of the storage period, FFA content of control, PS1 and CO1 samples were 1.73, 1.30 and 1.31 oleic acid %, respectively. FFA content of control group was detected to be 1.73 oleic acid % and it was similar to the value reported by Remisha et al., 2016.

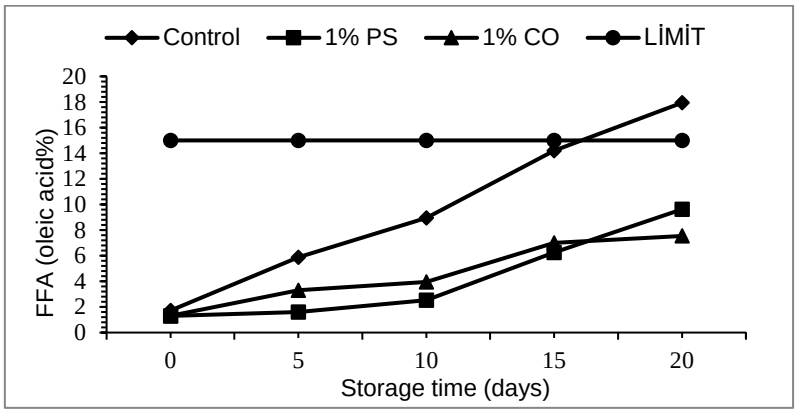

Figure 3. Changes in FFA content of crayfish samples treated with potassium sorbate and clove oil during storage at 2°C. C control, PS1 1% (v/w) potassium sorbate, CO1 1% (v/w) clove oil.

As it seen from Figure 3, there was a sharp increase in FFA content of control group but slight increase was observed in PS1 and CO1 group throughout the storage period. The increase in FFA content of control group was significantly ($p<0.05$) higher than those of crayfish samples treated with potassium sorbate and clove oil. There was a significant difference between the PS1 and CO1 group during the storage period ($P<0.05$). From day 0 to 15, the increasing rate of FFA content in PS1 group was slower than the control and CO1 group but between 15 and 20 days of storage, CO1 group had the lowest FFA content ($p<0.05$). At the last stage of period, amounts of FFA were detected as 17.94, 9.62 and 7.54 oleic acid % for control, PS1 and CO1 groups, respectively. While FFA content of control group exceeded the acceptable limit value of 15 oleic acid % (Korkut et al., 2007) at day 20, PS1 and CO1 group did not reach the upper limit value. In this study, FFA value of PS1 group (9.62 oleic acid %) was smaller than the value of 13.9 oleic acid % which was the value of 1% potassium sorbate dip treated mackerel reported by Remisha et al., 2016. Gandotra et al., 2014 reported that 5% potassium sorbate dip treatment significantly reduced the formation of FFA in silver carp fillet stored for 30 days.

According to FFA results, potassium sorbate and clove oil delayed oxidative and hydrolytic deterioration due to their antioxidant characteristics.

3.2. Microbiological Analysis

3.2.1. Total aerobic mesophilic bacteria (TAMB)

At day 0, initial TAMB of control, PS1 and CO1 were 3.21, 3.25 and 3.17 log cfu/g, respectively (Figure 4.). Between the 5th and 15th days of storage there was a significant difference between TAMB of samples ($p<0.05$). During the storage period, TAMB of all samples increased gradually and TAMB of treated samples were less than control samples ($p<0.05$). Least TAMB was observed in 1% clove oil treated samples throughout the storage period. Unlike the potassium sorbate treatment, clove oil showed the highest antimicrobial activity and inhibitory effect on aerobic spoilage bacteria. The antimicrobial activity of clove oil comes from its eugenol, oleic acids and lipid content (Nzeako et al., 2006). Probably, eugenol damaged the cell walls of bacteria by denaturating proteins and inhibited the growth of gram-positive and gram-negative bacteria by increasing membrane permeability (Nowak et al., (2012); Nunez and Aquino, (2012). After 10 days of storage, control group exceeded the acceptable limit value of 6 log cfu/g (ICMSF, 1986). At the end of the storage period TAMB of control, PS1 and CO1 samples reached

to 7.74, 5.31 and 4.85 log cfu/g, respectively. While control samples were microbiologically unacceptable, TAMB of PS1 and CO1 samples were below the limit value and there was no significant difference between TAMB of treated samples ($p>0.05$). Results showed that both potassium sorbate and clove oil were very effective in maintaining the microbial quality of crayfish during 20 days of cold storage. Reduction in TAMB of PS1 samples can be attributed to the antibacterial action of potassium sorbate on aerobic spoilage bacteria (Yesudhason et al., 2010).

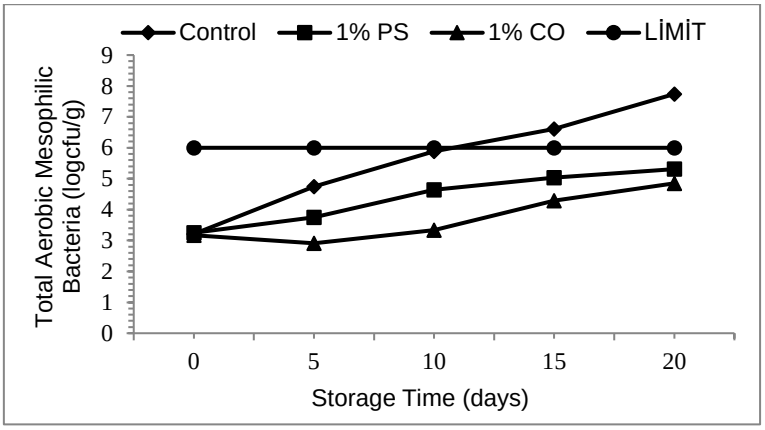

Figure 4. Changes in total aerobic mesophilic bacteria (TAMB) of crayfish samples treated with potassium sorbate and clove oil during storage at 2°C C control, PS1 1% (v/w) potassium sorbate, CO1 1% (v/w) clove oil.

Our results are in agreement with the findings of Remisha et al., (2016); Omojowo et al., (2009a). Remisha et al., (2016) reported that TAMB of 1% potassium sorbate treated Indian mackerel samples were lower than that of control samples during 18 days of cold storage. On the other hand, Omojowo et al., (2009a) found that microbial counts of potassium sorbate treated catfish samples were lower than citric acid treated catfish samples. Omojowo et al., (2009b) reported that potassium sorbate treated smoked catfish showed lowest microbial count than control and sodium metabisulphite treated samples during 8 weeks of storage.

3.2.2. Psychrophilic bacteria count (PBC)

Psychrophilic bacteria counts of control and treated samples are seen in Figure 5. The initial PBC were recorded as 2.96, 2.95 and 2.88 log cfu/g for control, PS1 and CO1 samples, respectively. The PBC showed an increase in all samples during storage period ($p<0.05$). Clove oil treated samples showed significantly lower ($p<0.05$) psychrophilic counts than control and potassium sorbate treated crayfish samples over storage period. The lower count in clove oil treated samples might be due to the antimicrobial action of clove oil. Similarly, Gandotra et al., (2014) reported that 5% potassium sorbate dip treatment was very effective in eliminating the growth of psychrophilic bacteria. Coban et al., (2018) reported that the addition of 1% clove oil inhibited the growth of psychrophilic bacteria in frozen trout fillets.

The PBC of control samples were high during 20 days of cold storage and control samples exceeded the acceptable limit value of 6 log cfu/g (ICMSF, 1986) after 10 days of storage. At the last stage of storage, PBC of control, PS1 and CO1 samples were 7.29, 4.96 and 4.75 log cfu/g, respectively and there was no significant difference among PBC of PS1 and CO1 samples ($p>0.05$). During 20 days of cold storage, PBC of PS1 and CO1 samples remained under the acceptable limit value.

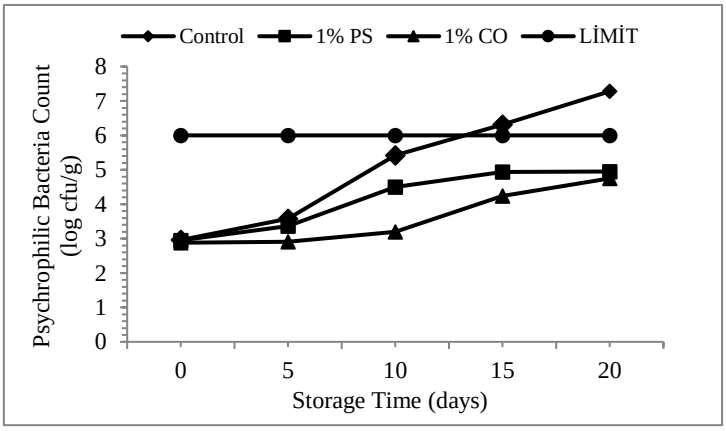

Figure 5. Changes in psychrophilic bacteria counts (PBC) of potassium sorbate and clove oil treated crayfish samples during storage at 2°C. C control, PS1 1% (v/w) potassium sorbate, CO1 1% (v/w) clove oil.

3.2.3. Yeast and mold count

Yeast and mold counts increased from the initial value of 2.16 to 4.34 log cfu/g for control, 2.19 to 3.75 log cfu/g for PS1 samples. As the storage days progressed, mold and yeast counts of CO1 samples decreased from 2.17 to 1.85 log cfu/g (Figure 6.). Clove oil treated samples showed lowest counts during 20 days of cold storage ($p<0.05$). Similarly, Coban et al., 2018 reported that clove oil treated rainbow trout fillets had significantly lower yeast and mold counts compared to control samples during six months of frozen storage.

Clove oil efficiently suppressed the growth of yeast and mold on chilled crayfish. The level of mold contamination in potassium sorbate treated samples could metabolize potassium sorbate thus reduced its fungistatic effect (Hasan and Abdolgader, 2012).

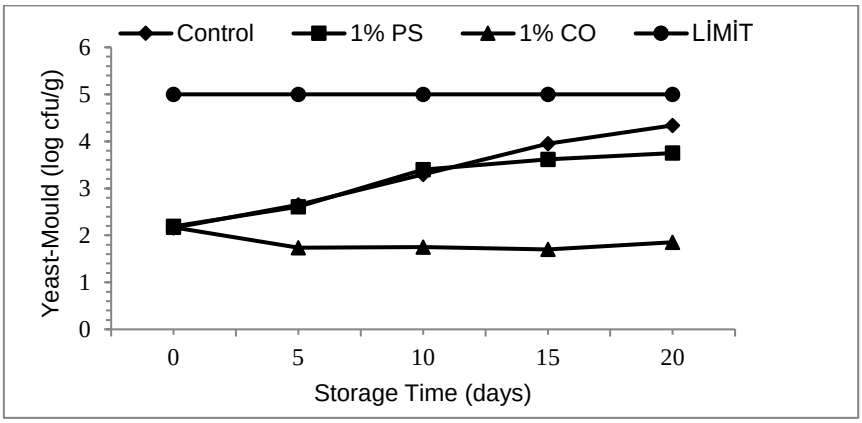

Figure 6. Changes in yeast and mold counts of crayfish samples during 20 days of storage at 2°C. C control, PS1 1 % (v/w) potassium sorbate, CO1 1 % (v/w) clove oil.

Between the zero and 10th days of storage, there were no significant differences between the control and potassium sorbate treated samples ($p>0.05$). At day 15 and 20, significant differences were observed between control and potassium sorbate treated samples ($p<0.05$). All samples were below the acceptable limit value of 5 log cfu/g (Stagnitta et al., 2006).

1% clove oil treatment was more effective than 1% potassium sorbate treatment in preventing growth of molds and yeast throughout the storage period. Clove oil showed strong antifungal activity due to its eugenol content. Eugenol content changed cell wall structures of yeast cells by increasing membrane permeability (Hyldgaard et al., 2012).

4. Conclusion

In this research, antioxidant and antibacterial effects of potassium sorbate and clove oil on the quality characteristics of crayfish samples stored at 2°C for 20 days were compared. Both 1% potassium sorbate and 1% clove oil application retarded lipid oxidation during 20 days of storage. Clove oil treated samples had significantly lower ($p<0.05$) psychrophilic counts (4.75 log cfu/g) then the control and potassium sorbate treated crayfish samples during storage period.

1% clove oil treatment was found to be potentially more effective than 1% potassium sorbate treatment on preventing growth of total aerobic mesophilic bacteria, psychrophilic bacteria, mold-yeast and on improving the quality of crayfish samples under cold storage conditions. Clove oil can be used in seafood preservation as a natural preservative agent.

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