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Full Length Research Paper

The effect of using a fungicide along with bactericide in the main soaking float on microbial load

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In the studies recently carried out on leather microbiology, a great deal of attention is paid to the fact that a considerable amount of fungi exist along with bacteria on the raw materials and in the pre-tanning processes and that they both have proteolytic and lypolytic activities. Because defects caused by fungi that mostly appear in tanning and post-tanning processes lead to drastic economical losses, it is important that fungi should be controlled from the initial steps of the manufacturing stages. To this end, bactericides with two different compositions (potassium dimethyl dithiocarbamate and quarternized compounds), and a fungicide (2-thiocyanomethylthio benzothiazole based) commonly used in Turkish leather industry were chosen. The bactericides were added into the main soaking float with and without different concentrations of fungicide. In each trial, liquor samples were taken at the end of the main soaking process and numbers of total aerobic mesophilic bacteria, the numbers of proteolytic and lypolytic bacteria, and numbers of total aerobic fungi, the numbers of proteolytic and lypolytic fungi were separately determined. When potassium dimethyl dithiocarbamate based bactericide at a ratio of 0.5% was used together with 2-(thiocyanomethylthio) benzothiazole based fungicide at a ratio of 0.5% in the main soaking process, it was found out that it was more effective against bacteria and fungi than when used alone. With the use of two antimicrobial agents in the aforesaid concentration, a synergistic effect appeared and whereas the number of fungi detected in the main soaking liquor decreased at a considerable level, all of the bacteria were controlled and so no bacterial growth occurred on the media.

Key words: Leather industry, soaking process, bacteria number, fungi number

INTRODUCTION

When the animal is alive, hides or skins resist against the penetration of microorganisms (Bailey, 1999; Birbir and Bailey, 2000). After they are flayed from carcass, their favourable composition (water 64%, protein 33%, fats 2%, mineral salts 0.5%, and other substances 0.5%) (Sharphouse, 1989) and arising of the flesh side make them more susceptible to microbial attacks (Cooper et al., 1972). Hides and skins may be exposed to the effects by microorganisms from both flesh and grain side. Orlita (2004) found that microbial deterioration characteristically begins from the flesh side in goat skins and from the grain side in cow hides. After being flayed, they are commonly preserved through salting to protect against possible microbial damages till the beginning of the production. Then, they are processed to obtain the resultant leather. “Leather tanning” is a general term for the numerous processing steps involved in converting animal hides and skins into final leather (Tissier and Chesnais, 2000). It contains wet processes and finishing operations (Aravindhan et al., 2007). Soaking conducted majorly in two phases as pre-soaking and main soaking is the first stage in the tannery process and is important for cleaning and rehydration of the hides and skins (Rangarajan et al., 2003). Moreover, some undesirable non-structural proteins are removed from them in this process (Thorstensen, 1993). In various studies carried out before, not only bacteria but also fungi have been detected in the salts (NaCl) used for conservation (Özyaral

Abbreviations: K-DMDC, Potassium dimethyl dithiocarbamate; QC, Quarternized compounds; TCMTB, 2-(thiocyanomethylthio) benzothiazole; cfu; colony forming unit.

and Birbir, 2005), in salted sheep skins (Bitlisli et al., 2004), in soaking and in other pre-tanning processes (Bilgi, 2007). It was shown by the researchers mentioned above that a great majority of the fungi in remarkable numbers have displayed proteolytic and lypolytic activities. In the course of main soaking, use of at least several times as much water as the own weight of the hides or skins, removal of the salt from them, the length of processing duration, the presence of foreign substances on the hides or skins are the parameters favouring the activities of microorganisms. Unless necessary precautions are taken during the soaking process, as a result of the microbial activities minor damages that can be understood with perceptible effects, such as putrid smell and matt, lustreless or blind sections in the grain; serious damages such as hair-slip, loose grain and reduced firmness, and heavy putrefaction such as pitting holes, putrefaction marks on the grain, loosening of the grain may appear (John, 1997). Further, numerous defects, such as looseness, weak fibre, weak grain, pitted grain, loss of grain layer, stains, uneven dyeing, uneven buffing and spueing may occur in the salted hides because of fungal effect (Özyaral and Birbir, 2005). In converting skin into finished leather, collagen which is the basic fibre component must be protected since many characteristics of the finished leather, particularly its durability, rely on collagen. Thus, bactericides with broad spectrum are widely preferred in the main soaking process to prevent bacterial attacks. However, fungi displaying proteolytic and lypolytic activities at a remarkable level on the raw hides and skins and in the pre-tanning floats should be taken into consideration. This study was conducted to determine how fungicide added in various concentrations along with two different bactericides widely used in the leather industry affect both bacterial and fungal population in the main soaking floats.

MATERIALS AND METHODS

Raw material

Wet-salted domestic sheep skins with average weight of 3 kg and average area of 6 sq. ft. were obtained from local suppliers and used for trials.

Bactericides and fungicide

Wide variety of bactericides and fungicides are used in the leather industry. The ones mostly preferred among by the leather manufacturers were chosen for the study. One of the bactericides is composed of potassium dimethyl dithiocarbamate (K-DMDC) and the other of quarternized compounds. The active ingredient of fungicide added together with the bactericides is 2-(thiocyanomethylthio) benzothiazole (TCMTB). Both bactericides and fungicide were used in commercial form.

Chemicals

Technical grade of chemicals and tap water were used to process the sheep skin and analytical grade of chemicals and distilled water to prepare the media.

Media

In order to find out the numbers of total aerobic mesophilic bacteria, the numbers of proteolytic and lypolytic bacteria in the soaking float, halophile medium (Anon, 2008) was modified and used. It consisted of 5.0 g KCl, 5.0 g $MgCl_2.6H_2O$, 5.0 g NH_4Cl, 5.0 g $MgSO_4.7H_2O$, 5.0 mL of trace element solution, 10.0 mL of 1% ferric-citrate solution, 30.0 mL of yeast extract solution (150 g/L), 30.0 mL of peptone solution (150 g/L), 10.0 g agar and 925.0 mL distilled water. Trace element solution (per litre of distilled water) was comprised of 1.0 mg $CuSO_4.5H_2O$, 220.0 mg $ZnSO_4.7H_2O$, 10.0 mg $CoCl_2.6H_2O$, 180.0 mg $MnCl_2.4H_2O$ and 6.3 mg $Na_2MoO_4.H_2O$.

So as to find out the number of total aerobic fungi present in soaking liquor, modified malt extract agar [30.0 g malt extract, 5.0 g peptone, 15.0 g agar, 10.0 g glucose, 1.0 g yeast extract, streptomycin (100 µg/mL) and 1000.0 mL distilled water] was used (Kis-papo et al., 2001). Both of the media were modified by using concentrations of NaCl at a ratio of 5%. Further, modified halophile medium and malt extract agar involving skim milk of 10% or Tween-80 (Riedel-deHaën 63161) of 2% were used to detect proteolytic and lypolytic microorganisms respectively (Sanchez et al., 2003).

Dilution of solution

Saline solutions containing NaCl of 5% was used for dilutions.

Processing the hides

Lab scale trials were carried out in the Leather Practice Unit of Biga Vocational College during April, 2008. In order to obtain more reliable results, the experiments were performed in parallel drums specially designed for laboratory use. The treatment methodology of the wet-salted sheep skins is shown in Table 1. In the experiment for the control group (C), no antimicrobial agents were used; in the other experiments (trial 1 - 8), additions of antimicrobial agents were applied as indicated in Table 2.

Samples

For microbiological analysis, liquid samples taken from each of the main soaking liquors were used. Without delay, the samples were transferred in an ice box (2 –6^0C) from Leather Practice Unit of Biga Vocational College to Microbiology Laboratory. Then, microbiological counting was done right away.

Microbiological analysis of samples

To determine the numbers of bacteria and fungi, spread plate technique was applied. 10 mL of each liquid sample was homogenized by using 90 mL of sterile saline solution. Serial dilutions up to 10^{-5} were prepared and then 0.1 mL of the diluted samples was spread all over the media. For bacterial growth, inoculated plates including NaCl of 5% were incubated at 41^0C for 72 h, whereas the plates were incubated at 27^0C for 3 weeks for fungal growth. Following the incubations, the colonies that emerged in the plates were counted and recorded. All experiments were done in duplicate.

RESULTS AND DISCUSSION

The numbers of bacteria and fungi obtained from the study are given in Table 3. For the control group, while bacteria range from 7.1×10^4 cfu.mL^{-1} to 2.0×10^6 cfu.mL^{-1}, fungi numbers vary from 3.1×10^4 cfu.mL^{-1} to 3.0×10^6

Table 1. Treatment methodology of wet-salted sheep skins (% based on the raw skin weight).

Process	%	Chemical	Temp. (^{0}C)	Duration (min.)	pH
Pre-soaking	500	Water	22	240	7.0
(No mechanical action during pre-soaking)					
Drain					
Main soaking	500 0.5 *	Water non-ionic emulsifier Antimicrobial agent(s)	22	 30	 7.0
Run on automatic (stop 55 min/run 5 min for 20 h)					
Drain					

*In the main soaking, antimicrobial agents were added as given in Table 2.

Table 2. The concentrations of antimicrobial agents (bactericides and fungicide) used in trials (% based on the raw skin weight).

Trials	Bactericides used (%)	Fungicide used (%)
Control	-	-
1	0.5 (K-DMDC)	-
2	0.5 (K-DMDC)	0.125 (TCMTB)
3	0.5 (K-DMDC)	0.25 (TCMTB)
4	0.5 (K-DMDC)	0.5 (TCMTB)
5	0.5 (QC)	-
6	0.5 (QC)	0.125 (TCMTB)
7	0.5 (QC)	0.25 (TCMTB)
8	0.5 (QC)	0.5 (TCMTB)

- : No any antimicrobial agent was used.

cfu.mL^{-1}. These data are significant due to the fact that they prove the existence of not only the bacteria population insistently discussed in the soaking process but also the fungi population in remarkable numbers. Moreover, the fact that 3.5 x 10^4 cfu.mL^{-1} proteolytic fungi were detected in the main soaking float proved that they should be taken into account from the initial steps.

When K-DMDC based bactericide was used alone at a ratio of 0.5% (trial 1) in the main soaking float, all of the bacteria and fungi numbers in question considerably decreased. Being used with TCMTB based fungicide at a ratio of 0.125% (trial 2), the bactericide decreased the numbers of the other microorganisms in question, except for numbers of total aerobic fungi, in limited numbers in comparison with its single use. When the concentration of the fungicide used with bactericide was 0.25% (trial 3), the numbers of the bacteria and fungi kept decreasing.

While the numbers of the fungi detected in the main soaking float decreased markedly, all of the bacteria were got under control and no bacterial growth was detected on the media when the concentration of the fungicide added with bactericide into the main soaking float is 0.5% (trial 4). Within the scope of this study, the lowest numerical values of all the experiments for fungi were obtained in this experiment. These values were determined to be 3.5x10^4 cfu.mL^{-1} for total aerobic fungi, 3.7x10^2 cfu.mL^{-1} for proteolytic fungi and 1.4x10^2 cfu.mL^{-1} for lypolytic fungi. According to the results, it can be readily concluded that joint use of these two antimicrobial agents (K-DMDC and TCMTB) in defined concentrations resulted in a significant synergistic effect.

When it was used alone (trial 5), QC based bactericide, similar to the case K-DMDC based bactericide was used alone, reduced the numbers of both bacteria and fungi at a considerable level in comparison with the control group. The bactericide was found more effective than K-DMDC based bactericide for total aerobic mesophilic bacteria (3.0 x 10^5 cfu.mL^{-1}) and proteolytic fungi (9.3 x 10^3 cfu.mL^{-1}). But the bactericide (QC) proved less effective for the other bacteria and fungi in question. When the bactericide was used with TCMTB based fungicide at a ratio of 0.125% (trial 6), it decreased the numbers of total aerobic mesophilic bacteria, proteolytic bacteria, lypolytic bacteria, total aerobic fungi and proteolytic fungi at limited level in comparison with its single use. When the ratio of fungicide used with bactericide is 0.25% (trial 7), other bacterial and fungal numbers except for those of total aerobic mesophilic bacteria kept decreasing when compared to trial 6, but this decrease remained limited. When concentration of the fungicide added with the bactericide was increased to 0.5% (trial 8), the number of total aerobic mesophilic bacteria was determined as 1.4 x 10^5 cfu.mL^{-1}, the number of proteolytic bacteria as 1.2 x 10^5cfu.mL^{-1}, the number of lypolytic bacteria as 1.5 x 10^4 cfu.mL^{-1}, the number of total aerobic fungi as 1.3 x 10^5 cfu.mL^{-1}, the number of proteolytic fungi as 1.1x10^3 cfu.mL^{-1} and the number of lypolytic fungi as 1.8 x 10^3 cfu.mL^{-1}, and in the experiments in which QC was used as bactericide (Trial 5 - 8), the lowest numerical values were obtained in this experiment.

Among the experiments carried out within the scope of the study, the lowest numerical values for bacteria and fungi were obtained in the trial 4 where K-DMDC based bactericide of 0.5% and TCMTB based fungicide of 0.5% were used together. The synergistic effect obtained in this trial was not attained with QC based bactericide and TCMTB based fungicide used together at the same ratio.

Table 3. The effect of fungicides used together with the different bactericides on numbers of some microorganisms in the main soaking float.

Microorganism	Numbers of microorganisms (cfu.mL^{-1})								
	C*	1	2	3	4	5	6	7	8
Total aerobic mesophilic bacteria	$2.0x10^6$	$7.9x10^5$	$1.6x10^5$	$8.6x10^4$	---	$3.0x10^5$	$1.7x10^5$	$1.7x10^5$	$1.4x10^5$
Proteolytic bacteria	$8.0x10^5$	$3.7x10^5$	$1.7x10^5$	$4.7x10^3$	---	$4.2x10^5$	$1.8x10^5$	$1.6x10^5$	$1.2x10^5$
Lypolytic bacteria	$7.1x10^4$	$1.3x10^4$	$1.2x10^4$	$1.5x10^3$	---	$3.9x10^4$	$3.3x10^4$	$3.0x10^4$	$1.5x10^4$
Total aerobic fungi	$3.0x10^6$	$3.0x10^5$	$3.0x10^5$	$1.8x10^5$	$3.5x10^4$	$7.1x10^5$	$3.0x10^5$	$2.5x10^5$	$1.3x10^5$
Proteolytic fungi	$3.5x10^4$	$9.7x10^3$	$1.9x10^3$	$1.4x10^3$	$3.7x10^2$	$9.3x10^3$	$3.2x10^3$	$3.0x10^3$	$1.1x10^3$
Lypolytic fungi	$3.1x10^4$	$7.8x10^3$	$2.0x10^3$	$1.3x10^3$	$1.4x10^2$	$2.0x10^4$	$2.1x10^4$	$2.0x10^4$	$1.8x10^3$

*The compositions of the control and the 8 trials are shown in Table 2.

Bilgi (2007) used only quaternary ammonium compound based bactericide of 0.4% in the main soaking process for domestic sheep skins and determined the numbers of total aerobic mesophilic bacteria as 5.8 – 7.2 x 10^4 cfu.mL^{-1}, the numbers of proteolytic bacteria as 3.4 – 5.4 x 10^4 cfu.mL^{-1} and the numbers of lypolytic bacteria as 2.4 – 5.3 x 10^4 cfu.mL^{-1}; the numbers of total aerobic fungi as 2.0 x 10^2 – 2.6 x 10^3 cfu.mL^{-1}, the numbers of proteolytic fungi as 3.0 x 10^2 – 9.0 x 10^3 cfu.mL^{-1} and the numbers of lypolytic fungi as 2.5 – 9.0 x 10^2 cfu.mL^{-1} on the media with different NaCl concentrations (0, 5 and 10%). The researcher stressed that it was important for the security of the next stages of the tannery process to get fungi along with bacteria under control from the first steps of the process.

In the study Meriçli Yapıcı et al. (2008) carried out by using salted hides and adding bactericide of 0.2% and fungicide of 0.2% into the main soaking float, they regenerated and reused the float of unhairing-liming process ten times and examined its microbial load in each batch. The researchers found out the maximum number of total aerobic mesophilic bacteria, maximum numbers of proteolytic and lypolytic bacteria and of aerobic spore-forming bacteria in the process with a pH value of 11 at least to be 3.9 x 10^2 cfu.mL^{-1}, 4.1 x 10^2 cfu.mL^{-1}, 5.4 x 10^2 cfu.mL^{-1}, 2.0 x 10^2 cfu.mL^{-1}, respectively, and maximum number of total aerobic fungi, maximum numbers of proteolytic and lypolytic fungi to be 2.8 x 10^3 cfu.mL^{-1}, 1.2 x 10^3 cfu.mL^{-1} and 3.5 x 10^3 cfu.mL^{-1}, respectively, on the media with different NaCl concentrations (0, 5 and 10%). As understood from the numbers of bacteria and fungi, although the researchers used both bactericide and fungicide in each soaking process in the study, they found that the numbers of fungi are a bit higher than those of bacteria.

In the previous studies carried out by many researchers, various raw materials and antimicrobial agents with diverse properties in different concentrations were used. Moreover, some of the processes where microorganism counting was accomplished have extreme pH values, especially for fungal growth. However, the fact that detected fungi are in serious numbers, further sometimes found more than the numbers of bacteria, is important. It is highly remarkable that a considerable amount of the fungi especially display proteolytic activities.

In this study, besides the bacteria with proteolytic and lypolytic activities in the main soaking float, it was also found out that fungi with the same activities exist in remarkable numbers. When each of the bactericides with two different chemical compositions (K-DMDC and QC) used widely in the main soaking floats were separately used together with TCMTB based fungicide, they generally decreased the numbers of bacteria and fungi in comparison with when used alone. Moreover, when TCMTB based fungicide and K-DMDC based bactericide were used together at a ratio of 0.5%, a significant synergistic effect was detected (trial 4). While the lowest numerical values of the whole study were obtained for fungi, no bacterial growth was detected in this experiment.

Undoubtedly, microbial problems encountered in the leather industry may vary from tannery to tannery because many factors, especially hides and skins used, affect the microbial load in the tannery. Therefore, microbial activities should be strictly observed not only in particular stages of converting the raw material into leather but also throughout the manufacturing process as a whole and necessary precautions should be completely taken not just for bacteria but also for fungi. By doing so, damages and defects that may adversely affect leather quality and accordingly economical loss can be avoided.

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