EFFECT OF *Yarrowia lipolytica* ON THE AROMA CHARACTERISTICS OF TURKISH FERMENTED SAUSAGES (SUCUK)

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Abstract – In this study, the effects of yeast strain *Yarrowia lipolytica* (*Y. lipolytica*) in combination with starter cultures (*Pediococcus pentosaceus*, *Staphylococcus carnosus)* on aroma characteristics of fermented Turkish sausages (sucuk) were investigated. The volatile compounds were analyzed using GC-MS coupled with Solid-Phase-Microextraction (SPME) method during 60 days of storage. The experiments were carried out with three batches of sausages containing control, starter culture and starter culture in combination with *Y. lipolytica* groups. Thirty seven volatile compounds including aldehydes, alcohols, acids, ketones, sulfur compounds and terpenes were identified in this study. It was determined that an important part of the volatile fraction in all sausage samples was composed of terpenes.

Key Words – Fermented sausages (Sucuk), Volatile compounds, *Yarrowia lipolytica*

I. INTRODUCTION

Sausage is a fermented meat product commonly consumed in Turkey [1]. In traditionally-produced sausages, fermentation is carried out under natural climatic conditions. However, modern meat industry have modified the traditional method of sausage production using starter culture and heat due to long processing time, variability in final product and dependence on natural climatic conditions [2]. Lactic acid bacteria and Gram-positive catalase-positive bacteria are the main starter cultures having technological importance in sausage fermentation. The characteristics of sausages produced with the addition of starter cultures and heat application are quite different from the naturally fermented ones with respect to taste and flavor. Currently, there is a much higher market share for the sausages produced with starter cultures and heat application than the naturally fermented sausages. Nevertheless, there is a strong demand for the naturally fermented sausages by consumers [3]. In this sense, different starter culture combinations have tried in order to obtain the flavor similar to those of traditional sausages. Studies carried out with different yeast species have indicated that they can positively contribute to flavor development and stabilization of red color due to their proteolytic, lipolytic activities and ability to degrade peroxides. *Y. lipolytica* is one of the yeast species frequently isolated from fresh beef and sausages. Due to its lipolytic and proteolytic activities, this strain could have a high technological potential [4]. The objective of this study is to investigate the effect of *Yarrowia lipolytica* on the aroma characteristics of Turkish fermented sausage "Sucuk" during 60 days storage at 4 °C.

II. MATERIALS AND METHODS

In sausage fermentation, the yeast strain, *Yarrowia lipolytica* (YB-618) was used. Three different batches of sausage production were produced in Pınar Meat Co. (İzmir, Turkey). The first batch was the control group without starter culture, in the second batch only starter cultures (*Pediococcus pentosaceus*, *Staphylococcus carnosus*) was added and in the third batch *Y. lipolytica* with starter cultures were blended with meat mixture. Sausages were analyzed at the first day sausage produced (day 1) and at the end of storage (day 60).

The changes in volatile composition of sausage samples extracted with solid-phase micro extraction (SPME) method were analyzed with GC/MS (Trace GC Ultra/ISQ, Thermo Scientific, U.S.A.) during storage. For this purpose, a fiber, coated with Divinylbenzene/ Carboxen/Polydimethylsiloxane and 30 m × 0.2 µm i.d. TR-5MS column (Thermo scientific, U.S.A.) with 0.25 µm film thicknesses were used. Samples, vacuum-packed and stored at -20°C for volatile compound analysis, were defrosted at 4ºC prior to analysis. Two grams of

minced samples were weighed into a 15 ml headspace vial, and a PTFE silicone septum was sealed with an aluminum crimp seal. Sample was equilibrated at 60ºC for 30 minutes. Then, fiber was inserted into headspace of the vial using SPME fiber holder. After 30 minutes, the fiber was inserted into the gas chromatography injector port and held for 5 minutes for desorption of absorbed molecules. The temperature of the injector port was 250°C. Carrier gas (He) flow rate was 1 ml/min. Oven temperature was programmed as: 40°C for 5 minutes then the temperature was raised to 165ºC (5ºC/min, held 5 minutes) to a final temperature 240°C (30ºC/min). Volatile compound fractions were expressed as percentage area.

III. RESULTS AND DISCUSSION

In this study, aroma characteristics of sausage samples were analyzed at the first day of sausage production (day 1) and 60th day of storage. The changes in quantity of volatile compounds are shown in Table 1. Thirty seven volatile compounds including aldehydes, alcohols, acids, ketones, sulfur compounds and terpenes were identified in this study.

Aldehydes are the volatile compounds formed by lipid oxidation. In the first day of sausage formation, control and starter culture added batches had lower percentage of aldehydes than *Y. lipolytica* added batch. Hexanal has an unpleasant fatty and potato like odor. The highest level of hexanal was recorded in *Y. lipolytica* batch. Nonanal is formed as a result of unsaturated free fatty acids oxidation and it has waxy and bitterish odor. The lower level was recorded in *Y. lipolytica* batch. During storage, percentage of aldehyde decreased in all groups. The addition of *Y. lipolytica* as starter culture reduced the production of aldehydes, which caused the unpleasant aroma formation in sausages. Acetic acid, 2-methylpentanoic acid, 2-hydroxypropanoic acid and hexanoic acid were the detected acids in sausage samples. Acids, which are formed as a result of carbohydrate and amino acid catabolism, gives cheesy odor to sausages [5]. Acetic acid that is formed by carbohydrate metabolism of homofermentative lactic acid bacteria and *Staphylococcus* spp. give sour taste [6]. Acetic acid content increased during storage. 2-hydroxypropanoic acid in other name lactic acid was produced by the fermentation of carbohydrates by lactic acid bacteria. At the beginning of the process, no lactic acid was detected in control and starter batches. A decrease was observed in lactic acid content of *Y. lipolytica* batch during storage. Because yeasts use lactic acid, the lowest levels were observed in yeast inoculated batch. Hexanoic acid, which gives unpleasant fatty cheesy and waxy odor, was the other acid detected in sausages. During storage, acid contents of sausages mostly came from acetic acid. Lowest content was observed in *Y. lipolytica* batch at the end of storage. Alcohols are the compounds that are formed by degradation of branched chain amino acids [5]. In some cases, they are produced as a result of decomposition of hydroperoxides by lipoxygenase enzyme. Ethanol is an alcohol produced by fermentation of sugar and has a slight odor. Ethanol level was higher in the yeast inoculated batch at the first day, but it decreased during storage. On the other hand, the literature on the relationship between yeast alcohol production and amino acid precursor availability in fermented sausages is scarce [7]. Acetone was the ketone detected in sausage samples. Ketones are formed by autoxidation and β-oxidation of free fatty acids, which are produced as a result of lipolysis. Starter batch had the lowest percentage of acetone. During storage, acetone content decreased. Allyl mercaptan, allyl sulfide, allyl methyl sulfide, diallyl disulfide, allyl trisulfide were the detected sulfur compounds, which originated from garlic, in sausage samples. There was a big difference between sulfur compound contents of yeast inoculated batch and other two batches. Sulfur compound level of *Y. lipolytica* inoculated batch decreased during storage. Terpenes are other volatile groups that have significant effect on sausage aroma. They are mostly came from the spices added sausage mix. Total of 13 terpenes were identified during storage. The greatest terpene percentage was recorded in *Y. lipolytica* inoculated batch and the lowest terpene contents were detected in control batch.

Table 1. Volatile compounds of sausage samples identified at the first day of sausage production (day 1) and at the 60th day of storage (area %)

Day 1				**Day 60**			
Identified Compounds	Control	Starter Culture	*Y. lipolytica*	Identified Compounds	Control	Starter Culture	*Y. lipolytica*
Ethanol	0.91±0.64	0.73±0.04	1.46±0.62	Ethanol	1.25±0.01	1.01±0.00	0.89±0.01
Acetone	0.70±0.09	0.72±0.06	0.89±0.33	Acetone	0.00±0.00	0.00±0.00	0.00±0.00
Allyl mercaptan	2.03±0.06	2.02±0.91	3.18±0.77	Allyl mercaptan	4.65±0.002	5.43±0.16	3.02±0.01
Acetic acid	0.75±0.00	0.65±0.09	1.08±0.15	Acetic acid	0.62±0.02	1.15±0.00	0.39±0.00
Ethyl acetate	0.00±0.00	0.00±0.00	0.00±0.00	Ethyl acetate	0.71±0.01	0.36±0.01	0.51±0.00
Allyl sulfide methyl	0.46±0.04	0.50±0.13	0.84±0.39	Allyl sulfide methyl	0.63±0.02	0.54±0.01	0.31±0.00
Pentanal	0.29±0.007	0.28±0.00	0.45±0.28	Pentanal	0.00±0.00	0.00±0.00	0.00±0.00
2-Butene	0.36±0.01	0.46±0.16	0.43±0.11	2-Butene	0.00±0.00	0.00±0.00	0.00±0.00
1-pentanol	0.48±0.28	0.38±0.16	0.43±0.07	1-pentanol	0.00±0.00	0.00±0.00	0.00±0.00
Hexanal	0.95±0.09	0.98±0.05	1.17±0.91	Hexanal	0.32±0.006	0.46±0.00	2.59±0.23
Allyl sulfide	0.39±0.09	0.36±0.06	0.38±0.07	Allyl sulfide	0.00±0.00	0.00±0.00	0.00±0.00
Pentanoic acid, 2-methyl	0.00±0.00	0.00±0.00	0.45±0.03	Pentanoic acid, 2-methyl	0.00±0.00	0.00±0.00	0.00±0.00
1-Hexanol	0.30±0.09	0.37±0.08	0.48±0.29	1-Hexanol	0.00±0.00	0.00±0.00	0.00±0.00
2-hydroxypropanoic acid	0.00±0.00	0.00±0.00	0.42±0.23	2-hydroxypropanoic acid	1.24±0.02	0.00±0.00	0.00±0.00
Styrene	0.55±0.33	0.85±0.04	0.35±0.18	Styrene	0.00±0.00	0.00±0.00	0.00±0.00
Dodecanal	0.82±0.11	0.27±0.03	0.81±0.07	Dodecanal	0.00±0.00	0.00±0.00	0.00±0.00
1-propene,3-bromo	0.00±0.00	0.55±0.07	1.38±0.48	1-propene, 3-bromo	0.00±0.00	0.00±0.00	0.00±0.00
Pinene	10.43±0.24	8.02±1.07	11.56±0.23	Pinene	8.37±0.001	7.74±0.03	13.79±0.14
Myrcene	6.01±0.13	6.32±0.49	4.87±0.11	Myrcene	6.10±0.03	6.47±0.12	22.09±1.12
Delta-3-carene	19.53±0.14	19.45±1.24	22.26±0.40	Delta-3-carene	0.00±0.00	0.00±0.00	0.00±0.00
Undecanal	0.21±0.03	0.23±0.04	0.35±0.21	Undecanal	0.00±0.00	0.00±0.00	0.00±0.00
Cymene	11.15±1.29	12.17±2.19	9.04±0.16	Cymene	5.94±0.08	4.56±0.01	7.42±0.13
Limonene	13.96±0.31	14.25±1.76	13.53±0.19	Limonene	6.04±0.16	6.10±0.30	3.98±0.12
Sabinene	0.48±0.01	0.53±0.08	0.41±0.09	Sabinene	0.45±0.007	0.54±0.01	3.60±0.03
Terpinene	3.42±0.07	3.58±0.37	2.75±0.75	Terpinene	3.27±0.02	3.47±0.02	0.81±0.00
Terpinolen	1.07±0.11	0.92±0.09	0.52±0.02	Terpinolen	1.65±0.003	1.61±0.02	7.14±0.00
Diallyl disulfide	7.5±0.41	8.25±0.57	9.42±0.26	Diallyl disulfide	7.68±0.21	9.63±0.33	2.17±0.00
Linalool	2.72±0.51	2.62±0.57	1.46±0.07	Linalool	3.86±0.06	5.84±0.24	0.24±0.00
Nonanal	0.00±0.00	0.35±0.09	0.23±0.04	Nonanal	0.00±0.00	0.00±0.00	0.00±0.00
Hexanoic acid	0.35±0.05	0.18±0.25	0.40±0.02	Hexanoic acid	0.51±0.006	0.49±0.01	0.00±0.00
Terpineol	0.40±0.18	0.43±0.03	0.28±0.07	Terpineol	0.39±0.003	0.34±0.01	0.63±0.00
Benzaldehyde	1.40±0.79	1.49±0.56	0.43±0.03	Benzaldehyde	0.99±0.004	1.03±0.00	0.64±0.04
2-decenal	0.23±0.05	0.26±0.04	0.38±0.03	2-decenal	0.00±0.00	0.00±0.00	0.00±0.00
Benzyl alcohol	0.76±0.08	0.77±0.01	0.50±0.06	Benzyl alcohol	0.00±0.00	0.00±0.00	0.00±0.00
Allyl trisulfide	0.29±0.06	0.29±0.09	0.34±0.01	Allyl trisulfide	0.00±0.00	0.00±0.00	0.00±0.00
Elemene	0.44±0.06	0.45±0.01	0.33±0.01	Elemene	0.61±0.001	0.72±0.01	2.07±0.01
Copaene	1.47±0.42	1.35±0.33	0.92±0.07	Copaene	2.05±0.02	2.51±0.00	7.87±0.23
Caryophyllene	6.74±1.19	6.78±1.48	4.05±0.29	Caryophyllene	8.49±0.41	8.64±0.12	1.44±0.01

IV. CONCLUSION

In this study, the effect of *Y. lipolytica* on the volatile compounds of fermented Turkish sausages was determined. Thirty eight volatile compounds were identified during storage. Volatile fractions of sausage samples were mostly composed of terpenes. Other identified compounds were aldehydes, alcohols, acids, sulfur compounds, ketones and aromatic hydrocarbons. The highest level of terpenes were detected in sausages inoculated with *Y. lipolytica*.

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