A RESEARCH ON THE USAGE OF RICE PRODUCED IN TURKEY TO PRODUCE SAKE

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ABSTRACT

A RESEARCH ON THE USAGE OF RICE PRODUCED IN TURKEY TO PRODUCE SAKE

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Sake has been processed from rice in Japan since the ancient times as a national beverage. It is consumed at the religional and traditional ceremonies. Polishing, washing, soaking, steaming, koji preparation, the preparation of moto as yeast starter and moromi fermentation are the main steps for sake brewing.

In the present study, it was aimed to designate features and the techniques of sake using short rice produced in Turkey.

In this study, short type rice (Karacadağ and Krasnodarsky-424), which were taken from local rice producers, were soaked in water after the polishing operation about 70 % remain. Then, *Aspergillus oryzae* was inoculated and solid-fermented starter koji was added. Then, *Saccharomyces cerevisiae* was inoculated and waited for fermentation for a certain time. Solid-liquid mix, which was obtained from fermentation, was filtered; sake, which was liquid part, was obtained. As a result, 12.65 and 13.8 % alcohol contents were obtained by using Karacadağ and Krasnodarsky-424 type short rice, respectively. When the results of sensory analysis were evaluated, it was observed that sake produced from Karacadağ type rice was acceptable.

Key Words: Sake, Koji, Turkish Short Rice, Karacadağ Rice, Krassnodarsky-424 Rice

ÖZET

TÜRKİYE'DE ÜRETİLEN PİRİNÇLERDEN SAKE ÜRETİLMESİ KONUSUNDA ARAŞTIRMA

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Sake, eski zamanlardan beri Japonya'da pirinçten işlenmiş ulusal bir içecektir. Dini ve geleneksel törenlerde tüketilir. Parlatma, yıkama, ıslatma, buharda pişirme, koji hazırlama, starter olarak moto hazırlama ve moromi fermantasyonu sake üretiminin başlıca adımlarıdır.

Bu çalışmada, ülkemizde üretilen kısa pirinçler kullanılarak sake üretim özellikleri ve tekniklerinin belirlenmesi amaçlanmıştır

Bu çalışmada, yerel b pirinç üreticilerinden alınan kısa tip pirinçler (Karacadağ ve Krasnodarsky-424), % 70 (kalan miktar olarak) parlatma işleminden sonra suda bekletildi. Sonra, *Aspergillus oryzae* aşılandı ve katı fermente-starter olan koji elde edildi. Daha sonra, *Saccharomyces cerevisiae* aşılandı ve belirli bir süre mayalanma için beklendi. Fermantasyondan elde edilen katı-sıvı karışımı süzüldü. Sıvı kısım olan sake elde edildi. Sonuç olarak, sırasıyla Karacadağ ve Krasnodarsky-424 tipi kısa pirinçler kullanılarak % 12,65 ve % 13,80 alkol elde edildi. Duyusal analiz sonuçları değerlendirildiğinde, Karacadağ tipi pirinçten üretilen sakenin kabul edilebilir olduğu gözlenmiştir.

Anahtar Kelimeler: Sake, Koji, Türk Kısa Pirinç, Karacadağ Pirinci, Krasnodarsky-424 Pirinci

To my daughter NESLİŞAH

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INTRODUCTION

1.1 Sake

Sake is an alcoholic beverage made of fermented rice. Sake processing is an essential area for the Japanese food industry. It has played an important role in Japanese life and culture for about thousand years, and the knowledge and skills required for the sake production have spread almost all over the country (Japan Sake & Shochu Makers Association, 2011). The history of sake dates back a thousand years, more than the discovery of chemistry. Sake brewing techniques and the skills of the masters has come to this day with experience (Kanauchi, 2013).

Allegedly, although it can be brewed almost anywhere in Japan, most famous sakes are brewed in areas with good water or high quality rice supply. Nada in Hyogo, Fushimi in Kyoto and Saijo in Hiroshima are some famous sake-producing regions (Japan Technical Information Services Corporation, 2006).

Although sake is a fermented beverage just like beer and wine, sake's brewing process is more complex. During sake brewing, polished rice grains are steeped in water before cooked by steam. Steamed rice is fermented with yeast, koji and water. In this process of brewing sake, koji mold enzymes saccharified the rice starch and the yeast ferments the liberated glucose into ethanol. The processes of saccharification by koji and fermentation by yeast proceed simultaneously in the same vessel. This simultaneous fermentation is called "multiple parallel fermentation". It is unique among the methods used for making alcoholic beverages compared with other alcoholic beverages; sake can be served both hot and cold (Furukawa, 2012). As a note, first stage of fermentation is a kind of solid fermentation. Modern scientific analysis methods allowed sake to be examined both in terms of the culture and the production techniques. Moreover, in the sake production, original techniques such as reaching higher than 18% high alcohol content with no distillation, low temperature fermentation, open vessel fermentation

with no sterilization and the fruity aroma creation were investigated (Kanauchi, 2013).

Rice cultivation came to Japan from China in the 4th century BC. The techniques of sake production from rice are thought to come from China at this time (Yoshizawa, 1999). In this period, it was observed that more productivity was obtained from the cultivation in the paddy fields than the cultivation in the dry field, thus increasing the need for labor for rice production. This situation allowed families to live together by settled in order (Yoshizawa, 1999).

In the periods when it was produced, rice was a valuable product for the people. Sake has become as important as boiled rice in production. So it is believed that sake was a sacred power, and it was thought that the drinkers shared this power. Moreover, sake drinking became an important part of the society. So, it has been a tradition for the king to serve sake at palace banquets for years (Yoshizawa, 1999).

The techniques from China contributed greatly to the development of the brewing using rice. In the 8th century, the court divided the sake sector into two as beer production and management. About ten different kinds of sake were produced for the banquets and gifts for the emperors and palaces. The production was about 45 kiloliters, annually. Rice and koji are added to the sake produced by rude filtration of moromi and the fermentation process is repeated many times. This led to have extremely sweet sake (Yoshizawa, 1999).

The production and sale of sake flourished after the 12^{th} century. The technology of fermentation improved from the fifteenth to the seventeenth century. The uses of a starter and increasing the amount of moromi, which are still in use today, have been developed as a new technique in these periods. White rice is used rather than brown rice. Sake is filtered from fermented moromi by pressing it into a filter bag. The making of over-sized wooden tubs was made possible by advanced woodworking techniques. During the Edo period (17^{th}-18^{th} century), large-scale brewing was due to these technical developments (Yoshizawa, 1999).

Heat treatment of sake was clearly demonstrated in a document about 1568 three hundred years before the discovery of pasteurization. In the second half of the 19th century, the introduction of the Western fermentation technology to the Far East and

the establishment of Japan National Brewing Research Institute led to a systematic approach to sake. Knowledge about yeast and mold has subscribed to the progress of today biotechnology and the microbiological industry (Yoshizawa, 1999).

1.2 Types of Sake

Industrially, sake classified into two main types: normal sake (ippan-shu or futsu-shu) and special named sake (tokutei meisho-shu). Tokutei meisho-shu, special named sake, is premium sakes distinguished by the degree to which rice is polished and the ingredients. There are eight types of special named sake;

- *Ginjo-shu*; is brewed from rice having less than 60 % polishing ratio, rice koji and pure distilled alcohol.
- *Daiginjo-shu;* is brewed from rice having less than 50 % polishing ratio, rice koji and pure distilled alcohol.
- *Junmai-shu;* is brewed from only rice and rice koji.
- *Junmai-Ginjo-shu;* is brewed from rice having less than 60 % polishing ratio and rice koji.
- *Junmai-Daiginjo-shu*; is brewed from rice having less than 50 % polishing ratio and rice koji.
- *Tokubetsu-junmai-shu*; is brewed from special varieties of rice having less than 60 % polishing ratio and rice koji.
- *Honjozo-shu*; is genuinely brewed from rice having less than 70 % polishing ratio, rice koji and pure distilled alcohol.
- *Tokebetsu*-Honjozo-shu; is specially and genuinely brewed from rice having less than 60 % polishing ratio, rice koji and pure distilled alcohol.

1.3 Sake Technology

Contrary to malt use in beer brewing or producing spirits, rice (polished) is used in sake brewing. Rice is polished in order to remove undesirable substances other than starch. The protein, oil and minerals on the surface of rice grains are removed by polishing. The polishing ratio is defined as the ratio of percentage by weight of polished rice to the original brown rice (Kanauchi, 2013). Changes in the some components of the processed rice grain with different polishing ratios are shown in Table 1.1 (Rose, 1977). The crude fat and ash contents decline most rapidly, despite the fact that the protein content declines progressively till 50 % polishing ratio, after which it remains practically constant. While the crude fat content changes depending on polishing, the lipid content (by hydrolysis) does not change with the increase in polishing ratio (Yoshizawa et al., 1973). The overall flowchart of sake production is shown in Figure 1.1.

In general, using 75-70% polished rice is feasible for sake brewing. However, the rate of polishing is reduced for more specific sake types. For example even 30 % polished rice is sometimes used for the grand grade sake (Kanauchi, 2013).

Table 1.1. Change in rice grain content depending on polishing rate (Rose, 1977)

Polishing Ratio (%)	**100**	**80**	**60**	**50**
Moisture	13.5	13.3	11.0	10.5
Crude Protein	6.55	5.12	4.06	3.8
Crude Fat	2.28	0.11	0.007	0.05
Ash	1.0	0.25	0.20	0.15
Starch	70.9	74.3	76.3	77.6

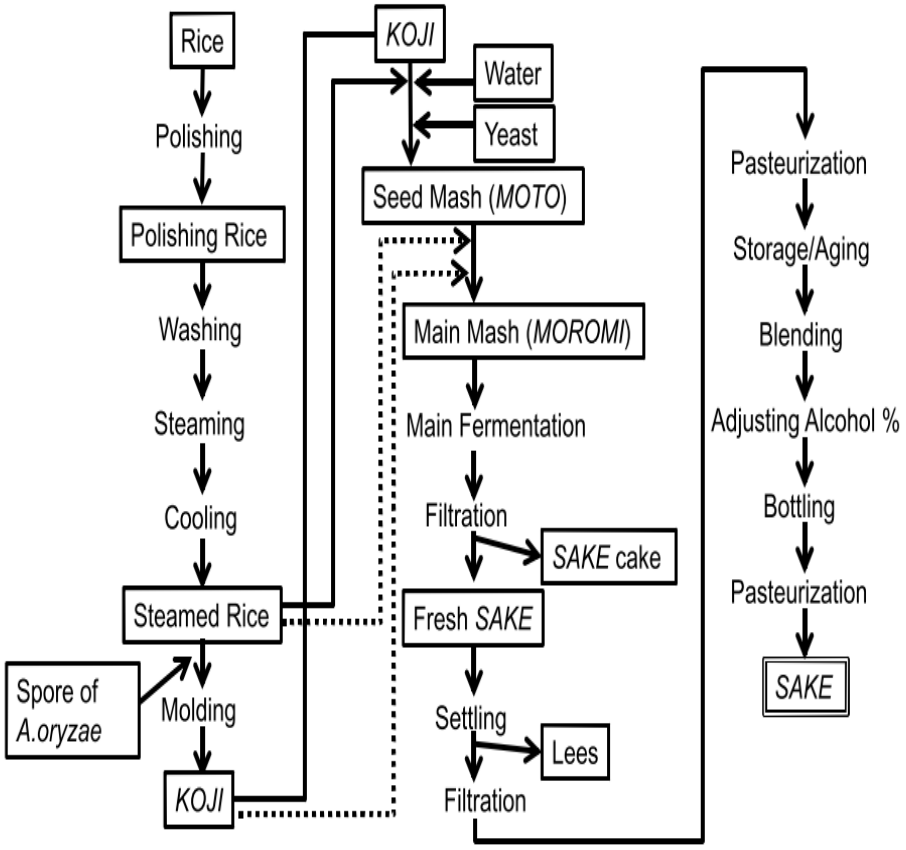

Figure 1.1. Sake Production flow chart (Kanauchi, 2013)

After polishing, sake brewing begins with washing rice and steeping in water before steaming. The grains are more polished when the rice grains strike together in water during washing. Approximately 1-3% of the total weight of the grain is removed from the surface components of the grain during processing (Rose, 1977). After washing, the rice taken into a vessel is then immediately soaked again. During washing and soaking operations, the grains absorb 25-30% water by weight. With the water draws out of the grains, it becomes easier for the heat to reach the grain center so that the starch in the grains is gelatinized faster. Absorption of water is highly critical for preparing appropriately steamed rice, managing koji making and fermentation. Rice variety and polishing ratio directly affect grains' water absorption capacity (Yoshizawa et al., 1973, Rose, 1977). Excessive polishing allows water to be absorbed further. When the sugar and potassium ions in rice pass into water during washing and soaking, (Yoshizawa et al., 1973; Kanauchi, 2013) metal ions

penetrate onto the grain (Rose, 1977). The excess water taken during the soaking is filtered for about 4-8 h before steaming (Kanauchi, 2013).

The steaming process leads to the denaturation of the proteins and the change of the starch to the α-form. Furthermore, it sterilizes the grains. Usually 30-60 min. is sufficient for steaming; however, the previous reports suggest that it is enough to steam 15-20 min. to denaturate the protein and change the starch for the Japanese rice varieties (Yoshizawa, 1995; Kanauchi, 2013). The grain absorb moisture by 7-12% of the starting weight, namely by about 35-40 % of the total water during steaming. The steamed rice is cooled nearly to 40 ^{o}C for proper koji production, and then rice is cooled to 10 ^{o}C for further process. Cooling process is done by machines by passing air through the screened belt (Yoshizawa, 1995).

Koji (rice koji) is the mold (*Aspergillus oryzae*) grown on a steamed rice medium. Because rice, a cereal, is mainly composed of polysaccharide starch, yeast cannot use it as an energy source as it is. The glucoamylase and alpha-amylase in koji break down rice starch into glucose, and make other resources in the rice available for yeast during the sake brewing process. This process is known as saccharification. Specifically acid protease and acid carboxypeptidase transform rice proteins into amino acids and peptides that have a great impact on yeast growth, sake flavor and aroma. In the wine production process, saccharification is not necessary, because glucose is already a significant component of grape juice. As a note, the wine production process is a single fermentation process (Furukawa, 2012).

The koji structure allows *Aspergillus oryzae* to develop in and over steamed rice. Thus, a wide variety of powerful enzymes have emerged. *A. oryzae* strains are sprinkled on steamed rice in wooden boxes at 34-36 °C and kept for 5-6 days. As a result of this process, a lot of spore formation is observed. Enzyme production depends on cultivation conditions. Generally, lower cultivation temperatures (around 30 °C) improve protease activity where higher cultivation temperatures (around 42 °C) develop amylases activity (Kanauchi, 2013).

The enzyme activities provided by koji are mainly as follows:

• The starch is cleaved by α-amylase, glucose is produced by glucoamylase and its activity regulates fermentation.

• Peptides are formed by the fragmentation of the protein by the acid protease. In addition, the acid protease assists in the action of amylase by breaking down the protein body that absorbs the α-amylase.

• Amino acids are composed by carboxypeptidase.

The longer the cultivation time, the more enzymatic activity occurs in the koji (Suzuki et al. 1956). Koji, made from steamed rice with high moisture content, contains nitrogenous substances and acids (Nunokawa, 1962). The flavor and the taste of sake are related to these substances. To grow koji molds, the steamed rice is first cooled to about 35 ^{o}C with the aid of a cooling apparatus, which is then transferred to large incubation chambers (koji-muro) where the temperature (26-28 ^{o}C) and humidity are controlled at the appropriate levels (Kanauchi, 2013).

For the proper koji, 60-100 g of seed mold is inoculated into 100 kg rice and this mixture is spread out in the middle of a wooden table. The temperature of the mix is around 31–32 °C at this process. When the mold activity starts in koji, the sweet chestnut-like moldy odor begins to appear in the steamed rice. The pile of the rice grains is mixed about 10-12 hours after incubation for a balanced control of temperature, humidity and growth. With mold growth, so more than 10-12 hours later, mold mycelia can be seen clearly as small white spots on the grains. At this stage, the material temperature rises to 32-34 ^{o}C. The material is transferred into wooden boxes, each with 15–45 kg of the grain. The bottom of the box is made of wooden lattice or wire mesh to control the rise in temperature and the moisture in the grain mass. On the other hand the thickness of the heap grains is also important to control the moisture content and temperature. The thickness of 8 cm at the beginning is reduced to 6 cm in the first mixing and 4 cm in the second mixing. After this stage, the material is mixed at 6-8 hour intervals and then spread again into the box. The temperature of the material increases to 40-42 ^{o}C after about 40 hours of incubation. The resulting micelles will penetrate into and surround the grain. At this stage, many enzymes, various nutrients and vitamins are formed for the development of sake

yeast. When the grains are covered with enough micelles, they are removed from the incubation room and left to cool on a clean cloth for mashing process. During koji making, acid-protease and α-amylase activities increase. Carbohydrates engender energy production for mold growth by decomposing finally to carbon dioxide and water (Kanauchi, 2013).

In sake brewing, the yeast starter is important for the main fermentation process. Yeast starter named as moto while main fermentation named as moromi. Moto serves to provide a clean and rich yeast crop to the main fermentation. It also provides the necessary lactic acid to prevent possible contamination in the early stages of the main fermentation. In the preparation of traditional yeast starter, lactic acid is produced by lactic acid bacteria in the mash. In the advanced method, pure lactic acid is supplemented to the mash at the initial stage of moto process. Recently, instead of preparing moto, compression yeast is used to ferment main mash safely. Seven percent of total rice is usually used to prepare moto (Kanauchi, 2013).

There are three main types of moto; Kimoto, Yamahaimoto and Sokujyo-Moto. The last one is usually used in the sake brew. The recipe of Sokujyo-Moto is: 100 liters of water are mixed with 30 kg koji and 70 ml of pure lactic acid (75%) is added to this mixture. A pure culture of sake yeast (105-106/g) is inoculated to the mash in which the temperature is 12 °C. Then, 70 kg of steamed rice is supplemented to the mixture and the mixture is sufficiently cooled to about 18-20 °C. The mash is stirred and agitated intermittently for 1-2 days, then it is heated same as in Yamahaimoto to increase temperature at a rate of about 1.0–1.5 °C/day. When the temperature rises to approximately 15 °C, sake yeast reaches the highest growth rate and fermentation begins. In order to shorten the cultivation time, mashing can be started at 25 °C and the moto temperature can be kept above 18 °C. In addition, Koon taka-Moto (hot-mashed Moto), one type of Sokujyo-Moto, is used by breweries. This mashing method is performed by subsequent inoculation of pure cultured sake yeast at 56–60 °C during several hours. The amount of water is raised to 150-160 liters per 100 kg rice used in order to prevent excessive sugar accumulation and high viscosity formation (Rose, 1977).

Moromi, the main mash, is fermented in an open fermentation system without special sterilization in an open container whose capacity ranging from 6-20 kiloliters. One

and half tons of polished rice was used for mashing one lot as standard. On the other hand, recently, larger tanks with 3-7 tones or sometimes over 10 tones have been used for mashing a lot. Water, koji and steamed rice are used to brew moromi mash.

For a usual moromi the raw materials used are given in Table 1.2. One of the significant characteristic of moromi mash production is the preparation of stepwise mashing as three steps. At first, water, koji and steamed rice are added to the moto. With this addition, the total acid and yeast population in the moto is diluted to about half. At the first mash, where the temperature is about 12 °C yeast increase gradually. Two days later, the yeast growth reaches 108/g by reaching the similar level with the moto. Almost twice as much material as the first addition is added as a second addition. Thus, the yeast population and total acids are diluted by about half too. The temperature reduced to 9-10 °C is suitable for the second addition. In the third and last addition, the materials are added in a greater amount than the first two additions (Kanauchi, 2013).

Table 1.2. Proportion of raw materials used in typical sake moromi (Yoshizawa, 1995)

	1st add.	**2nd add.**	**3rd add.**	**4th add.**	**Total**
Total Rice(kg)	140	280	890	160	2000
Steamed Rice(kg)	95	200	720	160	1580
Koji Rice (kg)	45	80	170	-	420
Water (liter)	135	250	1260	160	2460

The bulk amount of the main fermentation mash is approximately 14 times the mass of moto mash. This leads to diluted yeast cells. The reason for the gradual addition of the material is to prevent the development of wild microorganisms by decreasing the temperature of the mash in each addition. In sake brewing, at moromi, saccharification and fermentation occur simultaneously. Temperature control is so critical to keep these processes in balance. The small amount of sugar released from steamed rice and koji gradually fermented by sake yeast till alcohol content reach about 20 % (v/v). About 40% (w/v) of the sugar content in mash provides 20% (v/v) alcohol content formation. Sake yeast would not ferment alcohol in the mash if such a high concentration of sugars is supplied at once. As an alternative, as a

characteristic of sake brewing, it is the fermentation of the mash at low temperature (below 10-18 ^{o}C), which gives the balanced flavor and taste to the mash as well as the high alcohol concentration. The mash is agitated twice a day after the third addition of materials. With the effect of agitation, the mash density reaches maximum level after 3-4 days (Kanauchi 2013).

The foam formed during brewing is similar to soap suds. In addition, the foam slowly accumulates on the surface and forms a thick layer. At this stage, the indicator of healthy fermentation is a fresh fruit-like flavor. The fermentation is gradually strengthened by the warming of the mash. Viscous foam reaching to the mouth of the tank begins to form a deep layer (Taka-awa). Some breweries prefer to break Taka-awa with an electric mixer. The number of yeast cells increases by about 2.5 times, at this stage (Nojiro, 1959; Kanauchi, 2013). With increasing alcohol concentration, the foam become less concentrated and easily dispersed. Fermentation usually takes 20-25 days. Sometimes, it is preferred to add pure alcohol (30-40 %) to the mash in order to adjust final alcohol concentration to about 20-22 %. Usually 7-10% of the total steamed rice is added to the mash in the final stage of the moromi. In this way, the glucose is produced from the starch and the mash is sweetened by the saccharification effect of the koji in the mash (Kanauchi, 2013).

Upon completion of the alcohol fermentation, the mash is filtered and the sake and the solids are separated. For filtering, the mash is poured into approximately 5 liters bags placed in rectangular boxes. The mash is tightened by means of hydraulic pressure. After the filtration is complete, the solids are removed from the bag. Nowadays various automatic filter squeezers have been used for filtering process. The remaining solid from the squeezing process is called sake lees or sake cake. This cake, also called "*Sake Kasu*", contains starch, protein, yeast cells and various enzymes. In general, by processing one ton of polished rice, 200–250 kg of *Kasu* and 3 kiloliters of sake containing 20% ethanol are obtained. The sake, which is slightly turbid despite the filtration process, is clarified in a vessel by standing at low temperature for 5-10 days (Kanauchi, 2013).

After filtration, the soaked sake is allowed to stand for another 30-40 days and then pasteurized. The sake at the pasteurization process is passed through the helical tube heat exchanger at 60-65 °C for a short time.

Note that the pasteurization of Sake was made before the discovery of Pasteur. When pasteurization completed, sake is placed in closed containers for storing and active carbon can be added at this stage. Microbial infection is prevented by high alcohol content in the sake and pasteurization. The blended sake's alcohol is adjusted to 15-16.5 % (v/v) by adding water and by filtering through activated carbon, the flavor and taste is improved and its color and clarity are adjusted. In modern methods, after active carbon filtration, sake is passed through membranes or sheets having a plurality of pores of micrometer size. Thus, small particles, including microorganisms if any, are removed. This method allows pasteurization to be avoided and thus prevents quality degradation caused by heating. Sake rarely deteriorates; this deterioration is attributed to the formation of off-flavor and taste by diacetyl and acetic acid produced by Hiochi bacteria (Kanauchi, 2013).

Sake does not age for several years or more, it is usually stored and aged for a shorter period. Sake matures gradually during storage. The maturation process is probably the result of oxidation reactions and physicochemical changes. Sake alters and takes a softer taste. Considering the bottling time and the maturation rate, the storage temperature should be kept attentively at 13-18 ^{o}C (Kanauchi, 2013).

During aging, sake decays not only under the influence of amino-carbonyl reactions, but also under the influence of still unknown reactions. Furfurals produced in sake during aging are thought to add a cherry wine-like flavor to long-aged sake. In addition, the taste is soft and little affected by ethanol; this is due to the fact that ethanol and the water molecules are fluctuating in the sake during aging (Yoshizawa, 1995).

1.4 Koji

Koji rice is the cooked rice that has been inoculated with *Aspergillus oryzae*. The mold releases enzymes that ferment the rice by decomposing its carbohydrates and proteins. This process can also be applied to other grains like barley and legumes like soybeans.

Koji digests starches and proteins and breakdowns them into sugars and amino acids. This stage makes koji so special.

Rice is mainly composed of polysaccharide starch; yeast cannot use it as an energy source as it is (Furukawa, 2012). The glucoamylase and alpha amylase in koji break down rice starch into glucose and make other resources in the rice available for yeast during the sake brewing process. This process is known as saccharification.

While beer and wine are made by two separate processes, sake is fermented simultaneously with koji mold (*A.oryzae*) and yeast (*S.cerevisiae*), which makes the sake brew unique (Gogami et al. 2011). In addition to being unique, sake brewing is a complicated process. Enzymes produced by the koji mold *A.oryzae* saccharified the rice starch while the resulting glucose is fermented by the yeast *S. cerevisiae*. Koji mold, yeast strain and brewing conditions are common in determining the quality of the finished sake (Watanabe et al. 2011).

Since saccharification and ethanol fermentation take place at the same time in sake brewing, it is usual to obtain a very dense mash of steamed rice without accumulating a high level of sugar (Shimoi, 2008). High sugar accumulation prevents yeast growth. However, thanks to its unique parallel fermentation, the yeast cells in the mash reach the 20% (v/v) alcohol content among alcoholic beverages without distillation (Kotama, 1993).

It is suitable for the development of koji mold in and above the steamed rice grains. The mold accumulates various enzymes for sake production. Enzymes of about 50 kinds have been found in koji, the most important of which are amylases, α-amylases and saccharifying amylase play important roles in amylolytic action (Kanauchi, 2013). Additionally, proteases; acid proteases and alkaline proteases are found in koji. Enzyme activities of koji (unit/g) for α-amylase, glucoamylase, acid protease and acid carboxypeptidase are given by Yoshizawa (1999) such as 1200, 200, 3700 and 5100, respectively.

Specifically acid protease and acid carboxypeptidase transform rice proteins into amino acids and peptides that have a great impact on yeast growth, sake flavor and aroma (Furukawa, 2012).

Koji was produced under controlled conditions as explained. This stage was critical and the growth of koji mold should be controlled very well. Therefore, temperature

and relative humidity were controlled. Before koji processing, rice was polished, washed and then steamed.

In addition to its use in alcoholic beverages, koji is used in fermented food such as soybean paste (Miso) and soy sauce (Shoyu). Therefore, the food culture in the East is called a 'multi-fermentation culture' or 'moulds culture'. Far East climate characteristics make these methods possible (Furukawa, 2012). The moderately high-temperature humid zone from South-East Asia to East Asia enables the effective utilization of koji as a mould. Koji used for sake brewing is the same species (Kikoji-kin) used for Miso and Shoyu (Furukawa, 2012).

In the literature, there are some studies to investigate the cooking/steaming properties (Mizuma, 2013). Additionally, rice koji was observed using confocal laser scanning microscopy by Kojima et al (1999). In that study, an observational method using confocal laser scanning microscopy was developed. Thus, mycelia elongation and α-amylase production of koji mold (*Aspergillus oryzae*) in rice koji for sake making have been visualized. Specific antibodies against the mycelium of *Aspergillus oryzae,* glutelin (the major rice protein), and α-amylase from *Aspergillus oryzae* were prepared. Using these antibodies, rice koji samples were immunofluorescently stained and observed. Images of the mycelium of *Aspergillus oryzae*, glutelin and α-amylase were acquired separately and overlaid. It is revealed that α-amylase was produced around the mycelium from an early stage of growth, that the mycelium elongated along the cell wall of endosperm and that the amount of α-amylase increased with mycelia growth. The spatial distribution of the mycelium and its α-amylase production in rice koji were clearly visualized.

The digestion of proteins in steamed rice grains by sake koji enzymes under simulated sake mash conditions was analyzed by Hashizume et al. (2006) comparing the hydrolysis of steamed rice grains and heat-treated protein bodies isolated from different rice samples. The steamed rice grains pro-froundly affect the digestion of proteins in steamed rice grains by sake koji enzymes.

The activity of α-glucosidase in sake koji and its role during sake brewing is available in the literature (Morimoto et al. 1995).

1.5 Sake Microorganisms and Properties

Two microorganisms are used during sake brewing. *Aspergillus oryzae* is used as koji mold and *Saccharomyces cerevisiae* as yeast. In sake brewing, *A. oryzae* hydrolyze the starch into glucose by enzymes and *S. cerevisiae* produce ethanol from glucose simultaneously (Anzawa et al, 2013).

A.oryzae cultures on and within steamed rice grains. The mold provides 50 kinds of enzymes mainly amylases in koji required for the production of sake. Saccharifying amylase (exo-α-glucosidase; E.C. 3.2.1.20) and α- amylase (endo- α- amylase, E.C.3.2.1.1) have an important place in amylolytic action (Rose, 1977, Shimada et al., 1953, Kanauchi, 2013). Furthermore, some kinds of proteases such as acid-proteases and alkaline-proteases are also important enzymes found in koji. In sake mash, amino acids and peptides (oligo amino acid) are formed by the enzymes at low pH values (pH 3-4) (Rose, 1977; Kanauchi, 2013). Moreover, the food and nutrition growth of the yeast is supported by the amino acid or peptide. The enzyme treats implicitly, decomposing rice protein while integrating to an active site of the α-amylase (Siinoki, 1984; Kanauchi, 2013).

The taxonomy of *Aspergillus oryzae* was studied by Ahlburg and Matsubara (1878) and Cohn (1883). In detailed mycological study conducted by Wehmer in 1895 for the *A.flavus-oryzae* group, *A.oryzae* was identified as koji mold. The studies were slightly classified on variations in morphological and physiological properties (Rose, 1977; Kanauchi 2013). Murakami et al. showed that the mold used for making koji for sake brew is not *A.flavus*. In both types, they were distinguished on their authentic type culture based on mycological characteristics (Murakami 1968; Murakami 1972; Kanauchi 2013). Interestingly, koji molds used in the Japanese industry do not produce aflatoxin.

Sake brewing is a complex process that involves simultaneous saccharification of rice starch by enzymes produced by the koji mold *Aspergillus oryzae*, and fermentation of the resultant glucose by sake yeast strains, which belong to the family of budding yeast, *Saccharomyces cerevisiae*. Compare to other type of *S. cerevisiae* strains; sake yeasts produce much more ethanol during sake fermentation.

This feature is an important precondition for sake yeasts because quick and high-level ethanol formation allows for shortened fermentation periods, as well as inhibiting the growth of undesired microorganisms during sake brewing, in which open fermentation tanks are normally used. This has led to the fact that yeast strains with higher fermentation yields were selected as sake yeast (Watanabe et al, 2011).

Saccharomyces cerevisiae was discovered in 1830 by J. Meyen; it was named by E.C. Hansen in 1882. It has been not only used the sake brewery, but also in the brewery, the winery and the bakery (Rose 1977; Kanauchi 2013). Sake yeast is classified taxonomically in the *Saccharomyces cerevisiae* group (Kodama 1970; Kanauchi 2013). However, additional features that distinguish sake yeasts from other *S. cerevisiae* strains include acid tolerance, vitamin requirements, adaptation to anaerobic conditions and sugar osmophilic character (Takeda & Tsukahara; 1965, Nojiro et al., 1993; Kanauchi 2013).

During main mash fermentation, sake yeasts excessively produce foam. The foam formed during the usual main fermentation occupies one third of the capacity of the fermentation tank. Therefore, avoiding foam formation would be properly favorable for breweries to save up place occupied by the foam and increase the proportion of moromi produced. Foam formation is also considered to be caused by some large molecular weight compounds derived from steamed rice grains. On the other hand, nowadays, foam formation is thought to be related to the formation of foam on the yeast surface of existing proteins. Mutants with the same properties as the main yeast and which did not produce foam were obtained by Ouchi and Akiyama (Ouchi &Akiyama 1971; Nunokawa & Ouchi 1971; Kanauchi 2013). Foam-less mutant of sake yeast, a favorite strain of *Saccharomyces cerevisiae,* has become available for sake brewing (Shiomi et al., 2002; Kanauchi, 2013).

The metabolic processes of typical yeasts producing aromatic compounds are shown below. Iso-amyl alcohol and iso-butyl alcohol are the aromatic compounds classified as the higher alcohols. The two pathways used in the production of alcohol by the yeast are as follows.

1. $RCHNH_2COOH \Leftrightarrow RCOCOOH \rightarrow RCHO \rightarrow RCH_2OH$

2. $C_6H_{12}O_6 \rightarrow RCOCOOH \rightarrow RCHO \rightarrow RCH_2OH$

Lactic acid bacteria, which are the most important bacteria in sake brewing, are defined as below.

1. Bacteria are Gram positive. Their shapes are cocci or bacci.
2. They are facultative anaerobic bacteria.
3. They have no mobility and produce no spores.
4. Bacteria ferment glucose and producing more than 50% lactic acid per 1 molar of glucose.

The fermentation of lactic acids is of two types. One of them is homo type, 2 moles of lactic acid is fermented from 1 mol of glucose. The other is of the hetero type, 1 mol of lactic acid, 1 mol of carbon dioxide and 1 mol of ethanol are formed from 1 mol of glucose. *Leuconostoc spp*. (hetero-type cocci), *Pediococcus spp*. (homo type cocci) and *Lactobacillus spp*. (belongs to both types of bacci) are the ordinary lactic acid bacteria for food processing (Kanauchi, 2013).

In sake brewing, lactic acid bacteria are used in convectional seed mash. Thanks to the lactic acid bacteria in the production of kimoto, safe fermentation is ensured without sterilization. In convectional moto production, it is known that *Leuconostoc mesenteroides* as heterolactic acid fermentation grows the moto preparation earlier under extremely low temperatures of less than 5°C. *L. sakei* as a hetero-lactic acid fermentation grows in it (Kanauchi, 2013).

Commercial sake is uncommonly spoiled by Lactic acid bacteria. Bacteria that spoil the sake are called Hiochi bacteria and these bacteria are resistant to 18% ethanol concentrations in sake. These bacteria that grow in sake causes turbidity and unpleasant cheese-like odor (Kanauchi, 2013).

1.6 Sake Economy

Sake is one of the important sources of Japanese economy. It has high export potential as well as trade within the country. At the year 2017, 4,022,867 liters of sake were exported with the cost of approximately 175 million USD (Trademap, 2017). The most important importer is USA.

In the 2014 brewery year (1 July 2014–30 June 2015), 1627 breweries of all sizes were engaged in the production of sake (National Tax Agency Japan, 2017).

In Japan, the birthplace of sake, consumer preferences are changing, and alcoholic beverages are diversified into various kinds. In 2009 brewery year, the value of sake production (equivalent units 20% alcohol) is 469,378 kiloliters (a reduction of 4.9% compared with the previous brewery year), and this has reduced by approximately 9% compared with 2005 brewery year (522,281 kiloliters) (National Tax Agency Japan, 2010). On the other hand, in the USA and France, demand for sake is expanding briskly. In the UK, a sake section was established in the traditional international wine contest in 2007. Sake is becoming more familiar to people all over.

Table 1.3. Quantity of Sake exported by Japan (Liters) (Trademap, 2018)

2013	2014	2015	2016	2017
162.022.01	163.138.67	181.802.13	197.367.18	234.815.49

Table 1.4. Value of Sake exported by Japan (US Dollar thousand) (Trademap, 2018)

2013	2014	2015	2016	2017
107.834	108.660	115.789	143.472	166.569

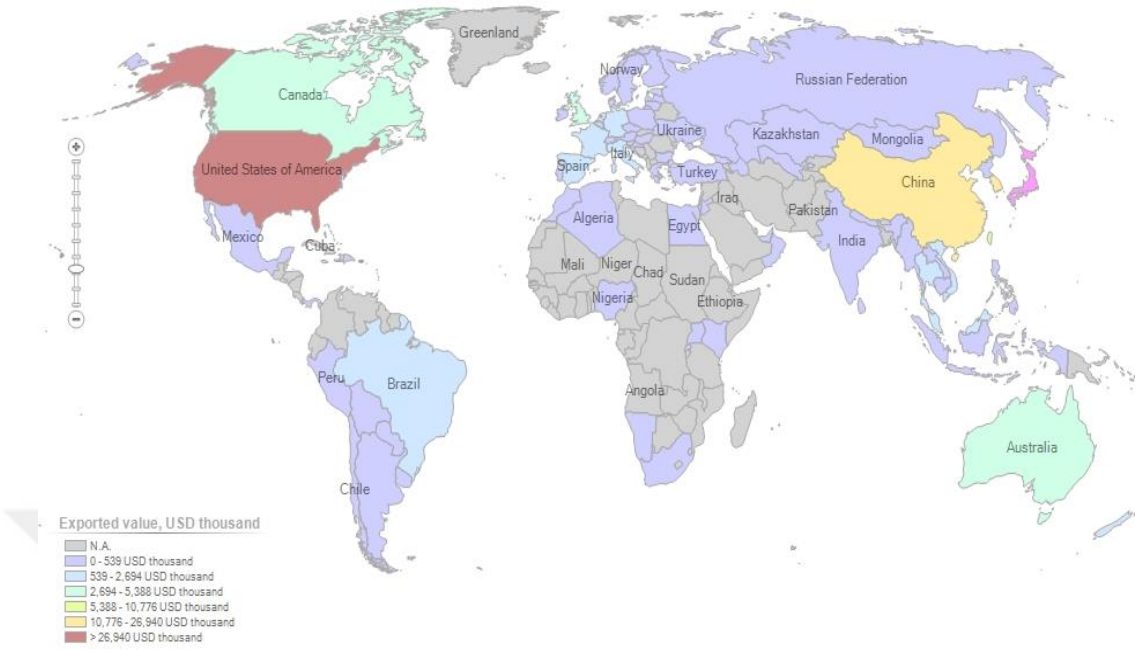

Figure 1.2. List of importing market for Sake exported by Japan in 2017 (Trademap, 2018)

1.7 Rice

Rice is an herbaceous plant species in the family of wheatgrass. After corn and wheat, it is most planted and is of great importance to feed for more than half of the world's population.

The cultivated rice plants, *Oryzae sativa*, thrive to about 1.2 meters in height. The leaves are long and flattened, and its panicle, or inflorescence, is made up of spikelet bearing flowers that produce the fruit, or grain.

Rice cultivation has been conducted for a long time with many societies such as China, India and Southeast Asia civilizations. In addition, archaeological evidence dating back to 7000-5000 BC shows that rice cultivation was carried out in Central and Eastern China at that time. Apart from the so-called plateau rice, the rice plant is grown in coastal plains, tidal deltas and lands underwater in the river basins of tropical, semi-tropical and temperate zones. The seeds are planted in ready bearings, and when the sets are 25 to 50 days old, they are transplanted to an arable field, or

paddy that has been enclosed by levees and submerged under 5 to 10 cm of water, remaining submerged during the growing season.

The harvested plant called rough rice or paddy is surrounded by the husk or hull. During milling, the husk and bran layer are separated and sometimes coated with glucose and talc to give the grains a bright appearance. Brown rice, processed to remove only the husks, contains small amounts of fats and about 8 percent protein and is a source of riboflavin, niacin, thiamine, calcium and iron. White rice, milled to remove the bran as well, is greatly diminished in nutrients. When the majority of the diet is with white rice, there is the risk of the disease of beriberi due to a lack of thiamine and minerals. In order not to decrease the nutritional value, the rice processed before milling is called parboiled rice and it is enriched by adding vitamin B and iron. The most suitable cooking method for rice is boiling. It is eaten alone and in a great variety of soups, side dishes, and main dishes in Oriental, Middle Eastern and many other cuisines.

The by-products of milling, including bran and rice polish (finely powdered bran and starch resulting from polishing), are used as livestock feed. Oil processed from the bran is used for both food and industrial. Broken rice is used in manufacture of starch and rice flour and in the brewing and distilling. Hulls are used for industrial grinding, fertilizer production, fuel, packing material and in the production of an industrial chemical called furfural. The straw is used for packing material, livestock bedding, feed, mats, garments, roof thatching and broom straws.

1.7.1 Rice Production Overall the World

Rice is grown in hundreds of countries. In the world, more than 700 million tons of rice is harvested from 158 million hectares of land annually. Four hundred seventy million tons of rice produced is milled. Ninety percent of global production is made in Asia with nearly 640 million tons of annual production. In addition, approximately 25 million tons are produced in the US and 19 million tons in Sub-Saharan Africa. Almost all rice in sub-Saharan Africa and Asia is grown in small farms (Ricepedia, 2018).

Yields range from more than 10 t/ha in intensive temperate irrigated systems to less than 1 t/ha under very poor rain field conditions. Small, and in many areas shrinking, farm sizes account for the low incomes of rice farm families. Compare to other crops, rice is yield in many conditions and grow up in a wide range of environment (Ricepedia, 2018).

The highest rice yields have traditionally been obtained from plantings in high-latitude areas that have long day length and where intensive farming techniques are practiced, or in low-latitude desert areas that have very high solar energy. Hokkaido in Japan, Northern California, Southwestern Australia, Italy, Spain and the Nile Delta provide the best examples (Ricepedia, 2018).

In Asia, rice production is higher than anywhere in the world and dominates the total crop production. Similarly, it is dominant in total food consumption (Ricepedia, 2018).

The vast majority of rice production in the world is made by China and India. India's harvest area is more than China, but because of irrigation, China's rice production is higher. Bangladesh, Indonesia, Myanmar Thailand and Vietnam, are the next largest rice producers. In the 2006 harvest, these countries produced more than 30 million tons paddy rice and accounted for 80% of world production (Ricepedia, 2018).

Although rice production and consumption is higher in Asia, rice is also one of the most important harvests in other parts of the world. In Africa, for example, rice has been the main staple food for at least 50 years in parts of western Africa and for some countries in the Indian Ocean. In these countries, the share of calories from rice has generally not increased significantly over time. However, in other African countries, due to its easy importation from Asia and the ease with which features can be prepared in urban life, rice has replaced the other ready food. In Africa production increased, consumption increased more rapidly, in this case the balance was achieved by imports. West Africa meets more than 40% of Africa's production and is the main producer sub-region. In terms of individual countries, the leading producers of paddy are Madagascar (3.2 million t), Nigeria (3.8 million t) and Egypt (7.0 million t) (Ricepedia, 2018).

In Latin America and the Caribbean, rice was a prior crop in the first half of the 20th century in the savannas of Venezuela, Uruguay, Colombia, Bolivia and Brazil and in forest border along the region. Today, rice is the most important source of calories in many Latin American countries including Haiti, Costa Rica, Dominican Republic, Peru, Ecuador, Panama, Guyana Suriname and the Caribbean nations of Cuba. However, because of the importance of wheat, maize, and beans in regional diets, rice is less dominant in consumption than in Asia. Brazil is the largest producer of paddy in the region and accounts for about 46% of the region's production with 11.6 million tons. Brazil is followed by Peru and Colombia with the production of 2.5 and 1.6 million tons respectively (Ricepedia, 2018).

In the US, California and the southern states near the Mississippi River are the most important production centers with an average of 9.0 million tons of paddy. The leading European producers are Russia, Spain and Italy. Australia was an important producer in the past, but in recent years its production has decreased significantly due to the repetitive dryness. In the last two decades, in Pacific islands rice consumption has increased rapidly. Rice, which is all imported apart from a small amount grown in Papua New Guinea, is displacing traditional starchy root crops as a major staple due to changing tastes, ease of storage and preparation and sometimes cost (Ricepedia, 2018).

1.7.2 Rice Types

Rice is usually defined as three types, depending on the length and shape of the grain, short grain, medium grain and long grain rice. Simply, short grain rice will be shorter and wider, whereas long grain rice will have a longer cylindrical shape.

The types of rice also determine the stickiness of rice. It is parallel to the length of rice. Long rice is less sticky in contrast to high sticky short rice varieties. This stickiness depends on starch components such as amylase and amylopectin. Amylopectin cause increase in stickiness and its content increases when rice size decreases.

The length of the long grain rice (Jasmine, Basmati etc.) is approximately three to four times the wide. Long-grained rice is separate, light and swollen when boiled because of its starch composition. Because of the low amylopectin content, they are less sticky rice types.

The length of the medium grain rice is almost equal to the width. When cooked, the grains tend to stick together. Calrose, Osmancık and Baldo are examples of this group.

The width of the short grain rice is twice as high as the size and sticky when cooked. In Turkey, Karacadağ and Krasnodarsky-424 are two main short type rice varieties.

Sticky rice, sweet rice, is grown mainly in Southeast and East Asia and used in many traditional Asian dishes, desserts, and sweets. It is especially sticky and often ground into rice flour, when boiled.

The rice has been applied a steam-pressure process before milling that gelatinizes the starch in the grain is called rough rice. The applied process causes light, puffy and separate grains when boiled. Converted rice is a type of parboiled rice that has been further pre-cooked, which ultimately allows you to whip up dishes of rice even faster.

Immediately after the harvest, the rice is brown and white when the layer of bran is removed. The unique pigments in the bran give the color of the rice (Red, Black and Purple). In these colored rice, the bran layer is not separated because it provides visual appeal and additional nutritional value.

The outer brown bran layer and the germ removed rice are called polished and known as white rice. They are also called milled rice.

1.7.3 Properties of Sake Rice

Rice is an essential element in sake brewing. The characteristics of sake are controlled by the quality of rice. Although the amount of protein is a small fraction compared with that of starch, protein is still important factor in sake brewing. Koji enzymes break down the proteins into amino acids and peptides. These amino acids and peptides not only provide nutrients for yeast growth but also provide flavor and

aroma components. Too much protein brings a zatsumi taste (Furukawa, 2012). This is poor and “lacking polish” taste.

The first major component of rice is starch, which approximately accounts for 78% (14% moisture) or 90% (dry) of milled rice by weight (Champagne, 2004). The second major component of rice is protein. It accounts for 4–11% of milled rice by dry weight (Champagne, 2004), which is affected by cultural conditions such as the amount of fertilizer used and changes in the temperature, as well as by rice variety (Champagne, 2004) and the degree of rice milling. Although the amount of protein is a small fraction compared with that of starch, protein is still an important factor in sake brewing. koji enzymes break down the proteins into amino acids and peptides. These amino acids and peptides not only provide nutrients for yeast growth but also provide flavor and aroma components (Furukawa, 2008).

Protein concentration is highest on the surface layers and decreases toward the centre of the grain (Champagne, 2004).Oil, minerals and vitamins, etc. are also most concentrated in the outer layer of the rice grain (Furukawa, 2012).

When observed at the distribution of protein concentration in the rice grain, it is seen that the amount of protein is high on the surface of the grain and decreases towards the center of grain (Champagne, 2004).

Oils in rice additionally cause “zatsumi” in the same way as does having too much protein. However, because of the outer layer is removed during polishing, these oils do not usually cause problem. Therefore the polishing ratio in the sake rice is 70% remain. The polishing the rice removes protein and oil found on the outer layer, but it is remaining starch-layer in the center to obtain superior taste.

On the other hand, the more the rice is milled, the less perceptible its cultivar characteristics become, and because the minerals and vitamins, which promote yeast health and good fermentation are also lost with high milling rates (Furukawa 2012).

Studies on the aroma in sake have shown that lipids and nitrogen sources in rice are effective in the flavor formation of sake (Yoshizawa, 1999). Yeast cells quickly incorporate fatty acids in the medium. Thus, aroma esters such as isoamyl acetate are formed during fermentation. Unsaturated fatty acids such as linoleic and oleic acid

substantially inhibit the formation of isoamyl acetate and resulting sake lacking the desired aroma (Ishikawa and Yoshizawa, 1984). Consequently, in terms of fatty acid concentration in the grain, the polishing operation is so critical in the quality control of sake.

In the study of Wakai, 1996, 92 Japanese varieties, which were obtained from daily food user and sake producers evaluated in terms of sake brewing suitability. They analyzed their physical and chemical properties and applied to a sake brewing test. Correlation analysis showed that some varieties which needed a long polishing time and were fermented slowly, had high amino acid content in sake, and left a certain amount of sake cake at the end of fermentation. On the other hand, the other varieties, which absorbed a large amount of water and digested well, were brewed to give sweet sake low in alcohol.

In 1997, another group (Yanagiuchi et al, 1997) also studied on the influence of grain type on the suitability of rice for sake brewing. Two types of grain were investigated. One of them is a white core in the center called shinpaku; the other is a non-core variety in the center called muhaku. In the production of koji, α-amylase production and micelle penetration rate in shinpaku type were higher than in muhaku type. However, no difference was found between the two types of grains in acid carboxypeptidase and glucoamylase activity (activities affecting control of the fermentation process). In the sake brewing test using steamed rice with shinpaku, total acid and obtained sake volume were higher. However, no significant difference was observed between the two grain types' fresh sake in terms of flavor components and other general components. So, this study also showed that the properties of raw material are important to produce good quality sake.

In another study, which was made by Okuda et al, 2016, the relationship between rice protein composition and nitrogen compounds (amino acids and oligopeptides) in sake was investigated. They found that the glutelin content of the highly polished rice used in the production of sake directly affected the nitrogen compounds in the sake. Thus, sake, which is made in rice species with low glutelin content, is generally observed to have mild taste. Generally, nitrogen compounds such as oligopeptides and amino acids contained in the rice varieties have a positive effect on sake taste.

There are also other studies about the low-glutelin rice varieties and their suitability's for the sake production in the different literatures (Mizuma et al. 2002, Furukawa et al. 2002). In the world, the finding new rice varieties are another issue. In the literature, a few study is available, for example Tamaki et al. (2005) studied on the varietal difference of polishing characteristics and suitability for sake brewing in "Hattani type varieties" of rice suitable for brewing original Hiroshima sake.

Another study was made by Tamaki et al. (2006) about the difference in the physical properties of white-core and non-white-core kernels of the rice varieties for sake brewing is unrelated to starch properties. Similar study was made by Wakai et al. 1996 on the influence of grain type on physical and chemical properties of rice for sake brewing. In their work, they mainly used rice with shinpaku and muhaku and observed differences between them. They observed differences in their physical properties such as 1000 grain weight, specific gravity, dynamic viscoelasticity, and total void volume. However, they observed no difference in their chemical properties. It was observed that shinpaku grains were more fragile than muhaku grains in the polishing process. In a digestion test, shinpaku grains had a higher Brix value than muhaku. However, differences in digestibility were found to be more highly significant among rice varieties than between the two types of grains.

The polished rice was used in the sake production. The polishing properties of white core grains fractioned from the same variety of rice were studied by Aramaki et al (1995). In their study, shinpaku and muhaku rice grains have been fully disintegrated by a shape-analyzing system basis of a principle of image analysis. Compared to the same type of rice grains with the same polishing rate, shinpaku grains were found to be softer than the muhaku grains and, as the polishing time increases, the shinpaku grains are more polished than the muhaku grains, and the hardness tends to decrease further. In the 120 minutes steeping process, the water absorption rate of both grains was about the same but the water absorption rate of the shinpaku was higher. At a polishing ratio of 50%, shinpaku grains had a lower loss of polishing ratio. It was also observed that, this tendency was the same in case of the practical polishing mill used in sake-making. Furthermore, it was ensured that the white core was observed on the surface of the vertically broken rice grains. The white-core showed a pair of arc shape and white-core was not appeared in the center of arc.

For sake brewing, rice is the one of the important raw materials. The characteristics of sake are controlled by the choice and combination of rice cultivars (Furukawa, 2012). Unlike grape aroma, which directly and strongly affects the taste of wine, rice does not have a characteristic and deep effect in the flavor of sake. Sake flavor depends on multiple factors during brewing (Yoshizawa, 1999).

The degree of milling for eating rice is usually about 90%; however brewing rice is usually polished from 70% in regular quality sake to 35% in excellent quality sake such as Daiginjo-shu. Such polishing of rice gives superior taste. On the other hand, the more the rice is milled, the less perceptible its cultivar characteristics become; and because the minerals and vitamins which promote yeast health and good fermentation are also lost with high milling rates, advanced techniques to supplement the mash for a clean fermentation process are needed (Furukawa, 2012).

Rice can be roughly classified into two types ordinary table rice and rice suitable for sake brewing (shuzo-kotekimai). Ordinary table rice, commonly used for eating, encompasses a number of sub-varieties, depending on the manner of cultivation and the region in which it is grown. Shuzo-kotekimai, sake rice, is used exclusively for sake production. Ordinary table rice can also be used to make sake, but, in general, sake made from shuzo-kotekimai has a better reputation for quality (Anon, 2005).

Rice varieties of non-wax short japonica, including varieties grown for cooking at both dinner and sake, can be used in the production of sake. Grains of sake type varieties generally have more suitable properties for sake brewing in terms of water absorbability, development of koji fungi and yeast, and the saccharification of starch than cooking-type varieties (Iemura & Fujita, 1982; Iemura et al., 1996; Okuda et al., 2006; Yoshizawa & Ogawa, 2004).

The physicochemical properties of rice used in sake brewing are known to affect sake quality. These are considered as water absorption capacity, 100 grain weight, moisture content and white haze (white core) in the grain center (Yoshizawa, 1999).

In Japanese varieties, the weight of 1000 grains is usually between 20 and 30 grams, and those over 25 g are generally considered suitable for sake brewing (Yoshizawa, 1999).

The void filled with starch granules is frequently seen at the center of the Yamadanishiki type. This structure shines and stands out as a white core. The grains, which have such a white core in its center, absorb water quickly and are well steamed. These varieties are particularly suitable for the production of koji, due to the growth of the *A.oryzae* mycelia on the surface of the white core cavity and on the surface of the grains (Yoshizawa, 1999). Seventy five percent polished white rice contains many protein granules in and around the endosperm cells. These proteins constitute 85 % of the total protein (Yoshizawa, 1999).

The water absorption capacity of the rice grain is extremely important for the gelatinization of the steamed rice and the growth of the koji mold. The moisture content of grains in the steeping and steaming processes is strictly controlled as water is absorbed quickly due to the loose structure of the endosperm (Mizuma et al., 2008; Tamaki et al., 2005; Yoshizawa & Ogawa, 2004). Chalky grains with a white core are generally considered to have undesirable properties for cooking and eating qualities, and grain breakage occurs in the tempering and polishing process (Lisle et al., 2000; Moldenhauer et al.; 2004, Yanagiuchi et al., 1996).

Additionally, the polishing properties of sake rice “Koshitanrei” for high-quality sake brewing were studied by Anzawa et al. (2013). At a high polishing ratio, breeding of a sake yeast mutant with enhanced ethyl caproate productivity in sake brewing using rice milled was investigated to see the effect of the polishing operation by Takahashi et al. (2017).

As explained previously, the polishing operation is used to remove unnecessary compounds e.g. protein and lipids. Additionally, this operation helps to absorption of water into rice kernel and reaching starchy center. The mechanics of water absorption was studied by Mizuma et al. and Mizuma et al. (2007, 2008). In these studies, the water absorption curve characteristics and structural properties of rice was used for sake brewing were investigated. The water absorption curve for rice suitable for sake brewing showed quantitatively sharper turn in the S-shaped water absorption curve than that of typical rice. The results showed that water penetration into the rice grain decreases the internal porosity and that the expansion during the water absorption process depends on the grain density. A characteristic S-shape water absorption curve for rice suitable for sake brewing was related to the existence

of an invisible shinpaku-like structure. Additionally, they found that rice has a monotonically smooth curve, whereas that suitable for sake brewing has an S-shaped curve (Mizuma et al 2006). As a note, the water migration and moisture distribution in rice grains used for sake brewing were also analyzed by Horiagne et al. (2014) by using magnetic resonance imaging.

1.8 Aim of Study

Sake is today economically important product in Japan, USA, Canada, Israel and European countries. Overall the world, its exportation and importation has been increasing. There are some local producers in USA and Canada. But the big exportation is made by Japanese companies.

Additionally, sake production know-how is a secret issue and its production details in the literature are very limited.

In Turkey, consumer prefers medium size rice. Short rice production (Karacadağ, Krasnodar) is made locally at limited quantity. Especially, Karacadağ rice is valuable local rice but its production is limited due to lack of value added product by using this variety. Additionally, Karacadağ is a special ancient place in Turkey, where is known as first place to plant wheat. Karacadağ is also near Göbeklitepe, where is also known as the place that the start of time for civilization.

The aim of this study is to research sake processing from the short rice varieties produced in Turkey to increase their economical values by using in sake as value added product. It is also aimed to obtain know-how of sake brewing method. The result of this study can be used to increase the value of Turkish short rice varieties and to increase knowledge about the sake production technique.

Additionally, the starting production of sake in Turkey can decrease the transportation cost of sake, comes from Japan to Israel and European countries. This product can cause a new investment for Turkey based on brewery product and to increase the utilization of short rice.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Rice

Rice samples (*Oryzae sativa*), harvested in 2014, Karacadağ and Krasnodarsky-424 were obtained from local producers in Diyarbakır and Tekirdağ, respectively. The varieties are short-size rice, which were selected as the most suitable varieties found in Turkey to produce sake.

2.1.2 Microorganisms

Aspergillus oryzae (ATCC, 1011 USA, CECT, 2094, Spain) and *Saccharomyces cerevisiae* (ATCC, 26422 USA, CECT, 10712, Spain) were obtained as freeze-dried form. In order to cultivation of *A.oryzae* and *S. cerevisiae*, Yeast Malt (YM) broth (ATCC, Medium 200, USA) was prepared as per the formula shown in Table 2.1.

Table 2.1. YM Broth/Agar formulation

Materials	**Amount**
Extract	3.0 gr
Malt Extract	3.0 gr
Dextrose	10.0 gr
Peptone	5.0 gr
Agar	20.0 gr
Deionized Water	1000 ml
pH	6.2 +/- 0.2

A. oryzae and *S. sake* were incubated at 30 ^{o}C for 24 hr. The cultures were lively obtained in broth and the enrichment was made by transferring the cultures to the fresh broth. When maximum growth was obtained, the purification was made by inoculating the cultures to the YM agars with streak plate technique.

2.1.3 Chemicals

The chemicals used in the experiments were obtained from Merck (Darmstadt, Germany).

2.1.4 Experimental Setup

Experimental setup for production of sake is given in Figure 2.1.

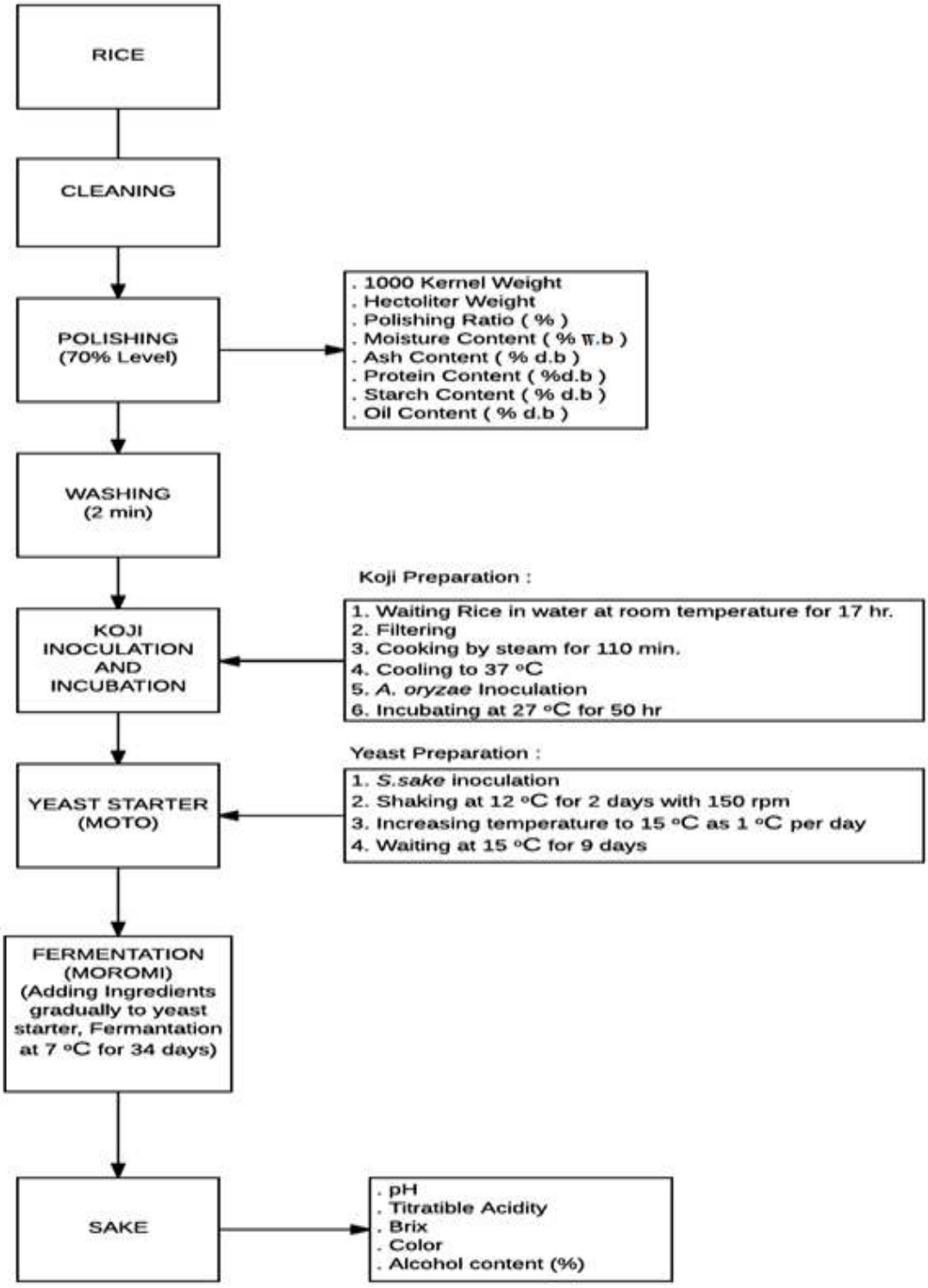

Figure 2.1. Experimental setup for production of sake

2.1.5 Sample Preparation

Rice samples were separated from dust, stone and foreign matters by using 3.5 mm screen and air aspirator.

2.1.6 Polishing

Rice varieties were polished till 70 % remain by using a modified polishing machine. Vertical emery stone type polisher was rearranged by changing cylindrical jacket screen. The aperture of jacket screen was 0.8 mm, and its surface was smooth (Figure 2.2). After polishing, the polished rice samples were washed with flowing tap water for 2 minutes. This washing helps to remove protein and starch powders found on the rice kernel surface remained from the polishing operation.

Figure 2.2. Emery stone type polisher with 0.8 mm jacket screen

2.1.7 Soaking

The washed samples were soaked in distilled water at the room temperature (25 ^{o}C) for 17 hours. The excess water was filtered after soaking. Soaking was applied to increase the moisture content of the rice kernels to around 30 % (w.b.).

2.1.8 Cooking

Rice samples were cooked by using steam for 110 minutes. Steam was obtained from boiled water in a kettle. Cooking was made for the gelatinization of rice starch. Soaked rice was put in a metal mesh and covered with filtered paper to prevent steam loss. Cooked rice was laid out on filter paper to cool to 37 ^{o}C.

2.1.9 Koji Making

Aspergillus oryzae culture was inoculated to the cooled rice to make koji. Inoculated rice was put in an incubator at 27 ^{0}C for 50 hours. The growing of *A. oryzae* was monitored at each 12-hour interval. It was decided that koji produced when the rice kernels totally surrounded by *A. oryzae* having a dark yellow color around kernels (Figure 2.3). Koji was stored at deep-freeze (-18 ^{o}C to be used for further process.

Figure 2.3. Koji produced for use in the study

2.1.10 Yeast Starter (Moto)

Moto (yeast starter) was produced by inoculation of *Saccharomyces sake* to the mixture of koji, steamed rice and water. According to the literature (Kanauchi, 2013) and practical production techniques, 20 gr koji was added to 45 gr steam cooked rice with 65 ml distilled water. In addition, 1-2 ml lactic acid solution (%75) was added to the moto to eliminate growing of wild microorganisms. Moto mixture was put into incubator-shaker at 12 ^{0}C for 2 days by shaking at 170 rpm. After 2 days, the temperature was increased at a rate of 1 ^{0}C per day to 15 ^{0}C and peak growing of *S. sake* was observed. Moto was kept at 15 ^{0}C for 9 days.

2.1.11 Main Fermentation (Moromi)

Moromi (main fermentation) was started by gradually adding moto (yeast starter) together with the addition of water, steamed rice and koji. Three stepwise additions were made, which is shown in Table (2.2).

Table 2.2. Ingredients Amount

Material	1st Addition (Day 0)	2nd Addition (Day 3)	3rd Addition (Day 7)
Koji (gr)	10	20	103
Steamed Rice (gr)	50	100	350
Water (ml)	60	118	600

After first addition, moromi was kept at 7 ^{0}C for 2 days as open fermentation. This temperature is the main fermentation temperature. As shown in Table 2.2, each addition was made by increasing the amount of materials with 3-4 day intervals. Fermentation took place 34 days at 7-10 ^{0}C and at each two-day intervals, the samples were taken for pH, alcohol content (%), titratable acidity (%, Lactic acid), Brix and color values (L*,a*,b*, YI) analysis.

2.2 Methods

2.2.1 Determination of Moisture Content

The moisture content (%) of polished rice was determinate by using AOAC 930.15 method (1999). The analyses were duplicated. Calculation was made by using following equation;

Moisture (%, w.b.) = (W1-W2) x 100 / W1

Where; W1: weight of sample (g) before drying

W2: weight of sample (g) after drying

2.2.2 Determination of Ash Content

The ash content (%, d.b.) of polished rice was determinated by using AOAC 923.03 method (1999). The analyses were duplicated for each sample. Calculation was made by using following equation;

Ash (%, d.b.) = (weight of ash) / (weight of dry sample) x 100

2.2.3 Determination of Protein Content

The protein content (%, d.b.) of polished rice was determinated by using Kjeldahl Method (AOAC, 1999, 920.87). The analyses were duplicated. About 1 g of sample was weighted to Kjeldahl tube. 12.5 ml Sulfuric Acid (H_2SO_4) three gram of potassium sulphate (K_2SO_4) and a small amount of cupper sulfate ($CuSO_4$) were added to the tube. The tubes were placed to the digestion unit for about 1.15 hr. 120 ^oC for digestion. After digestion, the tubes were placed into distillation unit to distillate NH_3. After distillation, the samples were titrated with 0.1 N HCl solution adding phenolphatelein as indicator. Used ml of HCl was recorded and calculation was made according to following equation;

Protein (% d.b.) = ((A-B) x N x 14.007 x 5.7) / W

Where A: Volume (ml) of HCl used for titration,

B: Volume (ml) of HCl used for blank titration,

N: Normality of HCl,

14.007: Atomic weight of Nitrogen,

5.7: The protein-Nitrogen conversion factor,

W: Weight (gr) of sample as dry basis

2.2.4 Determination of Fat Content

The fat content (%, d.b.) of polished rice was determinated by using Soxhlet Method (AOAC, 1999, 963.15). The analyses were duplicated. Three grams of sample was weighted to Soxhlet filter cartridge and placed into automatic Soxhlet equipment (Velp Scientifica, Ser 148, Europe). Calculation was made according to following equation;

Fat (%, d.b.) = (weight of fat / weight of sample as dry basis) x 100

2.2.5 Determination of Starch Content

The analysis of starch content (%, d.b.) was carried out by using Ewer's polarimetric method (ISO 10520, 1997). The polarimetric determination of starch content is based on the optically activity of starch. Due to the fact, that starch cannot be dissolved in water, hydrochloric acid is used. After dissolution, the sample needs to be clarified, filtrated and measured in a polarimeter. The optical rotation of all samples was measured at 20°C by using a sample cell of 200 mm optical path length. Results are presented as the mean of two replications with standard deviation.

2.2.6 Titratable Acidity

Titratable acidity was measured with titrimetric method (ISO 1843-2, 1997). To measure acidity of sake, 10 ml of sample is neutralized with 0.1 N Sodium Hydroxide. The value was multiplied by 0.075 to indicate the level of tartaric acid (g/100 ml). According to the standard, tartaric acid level is around 0.1-0.2 g/100 ml (Japan Sake and Shochu Makers Association and National Research Institute of Brewing, 2011)

2.2.7 1000-Kernels Weight

1000-kernels weight (gr) was calculated according to the method of Turkish Standards (TS 1136, TSE, 2007). 1000-kernels weight is a measure of seed size. It is the weight in grams of 1000 seeds.

2.2.8 Hectolitres Weight

Hectoliter-weight (kg/100 lt.) was determined according to the method of Turkish Standards (TS EN ISO 7971-1, TSE, 2012). Hectoliter-weight is a measure of the volume of grain per unit. It is usually expressed as kilograms per hectoliter and is a good indication of grain-soundness

2.2.9 Colour Measurement

The color was measured as CIE L*, a*, b* and YI (D65/10, C2.0) by using Hunter Lab colorimeter (Colorflex 45/0, HunterLAB, USA). L* indicates lightness, a* is the red/green coordinate and b* is the yellow/blue coordinate. Standard black and white plates were used to calibrate colorimeter before each measurement (L=93.01, a=-1.11, b=1.30 at D65). The color measurements were performed at room temperature (25 ± 2 °C).

2.2.10 pH Measurement

pH was measured by using a pH meter (Hanna Instruments, HI8314, Italy) at room temperature (25 ± 2 °C).

2.2.11 Brix Measurement

Brix was measured by using a refractometer (PTR 46X, Index Instrument Ltd., England).

2.2.12 Alcohol Measurement

Alcohol content (% ABV) was measured with a lab-scale electronic ebulliometer. (E060163, EON Trading, USA).

2.2.13 Sensory Analysis

Taste, odor and general acceptance of sake samples were evaluated by 25 panelists who are working/studying in Department of Food Engineering, Gaziantep University. The panelists had access to water to help cleanse their palates prior to proceeding to the next sample. The sensory evaluation was performed in a special sensory analysis room with appropriate temperature in open sitting. All samples were served at the same time on the same day. Panelists gave scores on a nine-point scale from 1 to 9 for odor, taste and overall acceptability of sake samples. Sensory analysis form is given at appendix A.22.

2.2.14 Statistical Analyses

The analysis was carried out as 2 replicates for all determinations. The mean and standard deviation of means were calculated. The data were analyzed by one-way analysis of variance (ANOVA). Duncan test (α=0.05) were applied to determine difference between homogeneous group. A multiple comparison procedure of the treatment means was performed by Pearson correlation test. Statistical Analyses were carried out by using IBM SPSS Statistics (v22.0.0, 2014, IBM Corporation, New York, USA).

CHAPTER 3

RESULTS AND DISCUSSION

The history of sake, which is very old, is described in kojiki and nihonshoki. The brewing technology in Asia was well established compared to Europe, and the Song dynasty in China had discovered the low temperature required for brewing about 1865 when 100 years ago from Pasteur (Yamashita, 1997, Sato & Kohsaka, 2017). Japan acquired brewing technology from China in about 1570 (Nishiya, 2011, Sato & Kohsaka, 2017).

The evolution of sake was slow in the Christian missionary from the 16th to the 19th century, which was due to the technology's production of immature flavors (Yoshida, 1993, Sato & Kohsaka, 2017).

The prestige and desire for the high-quality sake are quickly increasing. These improvements are thanks to rice polishing technology, better malt and yeast and the know-how for producers with increased access to state of the art scientific knowledge and new production technologies (Sato & Kohsaka, 2017).

These properties become clearer when compared to wine in sake evolution. The wine production process is simpler than the production of sake, and production methods are almost unchanged. For example, Bordeaux Primary School, which was rated by Napoleon III in the mid-19th century, is now produced under the same system as it is now (Sato & Kohsaka, 2017).

In this study, sake was produced from local rice, which were short types. One of them e.g. Karacadağ was a special, which is produced in ancient Karacadağ region. Karacadağ region is found in Mesopotamia and it is known as the origin of wheat overall the world. It is also found Fertile Crescent. Karacadağ rice grows only in this region at very low quantity. It is short short-rice but it is expensive due to its special taste. Therefore it was selected one of the raw material in this study. The other type was Krasnodar, which its origin is Russia. It was also planted in Minor Asia

(Anatolia). It is short-rice and its consumption is very low for daily-table use. Its flavor/aroma is known as neutral.

3.1 Physical and Chemical Properties of Raw Materials

The properties of rice types (Karacadağ and Krasnodarsky-424) used in this study are shown in Tables 3.1 and 3.2, respectively.

Table 3.1. The properties of Karacadağ rice type used in the experiments (polished at ~ 70 % remain ratio)

Properties	Karacadağ Type
Moisture content (%. w.b.)	13.73 (±0.16)
Ash content (%, d.b.)	0.23 (±0.13)
Protein content (%, d.b.)	5.11 (±0.11)
Fat content (%, d.b.)	1.14 (±0.00)
Starch content (%, d.b.)	60.33 (±1.06)
Hectoliter-weight (kg/100 L)	86.42 (±0.22)
1000-kernels weight (g, d.b.)	22.54 (±0.07)

Table 3.2. The properties of Krasnodar rice type used in the experiments (polished at ~ 70 % remain ratio)

Properties	Krasnodar Type
Moisture content (%, w.b.)	14.27 (±0.02)
Ash content (%, d.b.)	0.49 (±0.15)
Protein content (%, d.b.)	6.33 (±0.05)
Fat content (%, d.b.)	0.29 (±0.01)
Starch content (%, d.b.)	69.38 (±1.08)
Hectoliter-weight (kg/100 L)	87.19 (±0.09)
1000-kernels weight (g, d.b.)	23.61 (±0.11)

3.1.1 One Thousand Weight of Rice

It was stated that the rice type having 1000 grain weight between 20 and 30 gr is suitable for sake brewing (Yoshizawa, 1999). The one thousand grains weights used in this study were 22.54 (±0.07) g for Karacadağ and 23.61 (±0.11) g for Krasnodar types. Those weightings were suitable for the sake brewing as it is also reported in many texts in the literature (Yoshizawa, 1999; Kanauchi, 2013).

3.1.2 Hectoliter Weight (Bulk Density) of Rice

Theoretically, the bulk density of milled rice is significantly affected by moisture content. It is also dependent on the grain particle size (long, medium or short grain). The bulk density of Karacadağ rice variety was 86.42 (±0.22) kg/100 L while Krasnodar type was 87.19 (±0.09) kg/100 L. Their bulk densities were nearly the same.

3.1.3 Moisture Content of Rice

The rice has a moisture content of 14-15% after harvesting. The high moisture content of the grains results in breakage during the milling process and thus a reduction in milling recovery. Thanks to milling, the moisture content of the grains decreases due to heat generation and water evaporation. During the washing and soaking prior to steaming, these processes contain much water; the rice with low moisture content draws more water. In this case, handling and making koji becomes difficult. According to the result of this study, Karacadağ type having the moisture content of 13.73 (±0.16) % (w.b.) and Krasnodar type having the moisture content of 14.27 (±0.02) % (w.b.) were suitable for the sake brewing.

3.1.4 Ash Content of Rice

When the organic matter and water are removed by burning in the crucible, the remaining inorganic part is ash. The minerals such as potassium, phosphate and magnesium have a positive effect on the quality of the sake, making it easier for the yeast to reach the amino acid, accelerating the fermentation and encouraging the growth of mold and yeast (Yoshizawa, 1999). Additionally, ash content shows the

remained bran content on the surface of the rice. The ash contents of Karacadağ and Krasnodar rice were 0.23 (±0.13) % (d.b.) and 0.49 (±0.15) % (d.b.), respectively.

3.1.5 Protein Content of Rice

According to the literature, protein content (Nx5.95) of the brown rice varies from 7 to 9%, depending on the type of rice, cultivation conditions (such as fertilizer used and temperature changes). Protein structure is mainly consists of glutelin. The ratio of gluten content to other proteins increases to the interior of the kernel. Polishing causes reduction in protein content. The protein content of 75% polished rice is usually between 5-6%. In the present study, the protein content of Karacadağ type was measured as 5.11 (±0.11) % (d.b.) while Krasnodar type was measured as 6.33 (±0.05) % (d.b.).

During the main fermentation, peptides and amino acids that give full and heavy taste to sake result from acid protease and carboxyl peptidase enzymes hydrolyzing the protein in koji. However, in case of excessive amounts of these compounds, hard taste usually occurs, the color deepens and quality deterioration occurs during storage. Certain amino acids such as leucine, isoleucine and valine are assimilated with yeast to form higher alcohols such as isobutanol, 2-methyl butanol and isoamyl alcohol (Yoshizawa, 1999). Since consumers prefer sake with a simple and light taste, efforts should be made to reduce protein content of steamed rice and denature proteins.

3.1.6 Starch Content of Rice

Starch covers the vast majority of rice and has the majority of carbohydrate content. However, there is trace amount of glucose sucrose and fructose in the grain. Cellulose and hemicelluloses are found in the endosperm cell walls. In rice varieties suitable for sake production, these cellulosic compounds are less than normal varieties (Yoshizawa, 1999). Sake rice typically has a lot of starch at the center of the grain called "shinpaku". Because, shinpaku is white and very soft, it easily absorbs the koji mold, which converts the starch into sugar. In terms of suitability of the varieties, Karacadağ type had a starch content of 60.33 (±1.06) % (d.b.) and

Krasnodar type had 69.38 (±1.08) % (d.b.), which these amounts make them appropriate for the sake brewing.

3.1.7 Fat Content of Rice

Rice containing low fat content is desired for the quality of sake brewing. Brown rice has 2.5-3 % fat. Milling reduces the fat content to the level of 1 % (Yoshizawa, 1999). The rice used in the sake brewing should be polished min 70 % polishing ratio. The more polished rice, the more quality sake brewed. Polishing removes the fat, which is present on the surface layer of rice like protein. The rice varieties used in this study were polished as 70% remain polishing ratio it was obtained that Karacadağ type had 1.14 (±0.00) (%, d.b.) fat content while Krasnodar type had 0.29 (±0.01) (%, d.b.).

3.2 Comparison of Karacadağ and Krasnodar Rice

Thousand weights of rice grains is an important physical parameter of sake rice. The higher thousand weight of rice grain used for sake brewing, the more suitable for the sake production. Thus, in terms of thousand weights, Krasnodar type was more suitable than Karacadağ type.

As discussed earlier, during the washing and soaking prior to steaming, these processes contain much water; the rice with low moisture content draws more water. In this case, handling and making koji becomes difficult. When compare both rice varieties used in this study, it was obtained that Krasnodar type seems more suitable than the other for the sake brewing.

As explained previously, the ash content in rice indicates the presence of minerals such as phosphate, potassium and magnesium, which are desirable for the quality of the sake brewing. Karacadağ and Krasnodar types' rice both were suitable for the sake brewing in term of included the ash content.

As it remarked many times in the literature, the excess amount of protein leads to the deterioration of aroma and flavor of sake (Yoshizawa, 1999; Kanauchi, 2013). According to the results, Karacadağ type contains less amount of protein than

Krasnodar type. Therefore, Karacadağ type rice was more suitable for the sake brewing in terms of the protein content.

Starch is the main raw material of alcoholic fermentation. It is converted to the fermentable sugar during koji making. Sake fermented from rice with high starch content has higher alcohol content. At that point, Krasnodar type rice had higher starch content than Karacadağ type, therefore it resulted higher alcohol content in this study.

According to the literature, fat content causes the deterioration of flavor and aroma of sake. In the study, Karacadağ type had more fat content than Krasnodar type.

According to the literature, other components such as proteins and lipids in the raw material, except starch, are unnecessary for the sake production; therefore these compounds should be as little as possible. In this study, both raw materials supplied these conditions.

3.3 Properties of Sake Produced from Different Rice Varieties

After koji stage, the second stage was carried out. In this stage, the main fermentation (*S.cerevisiae*) was made to produce sake by producing alcohol.

The effect of rice varieties on sake quality has been the subject of research for a long time, but the more dominant effect of the brewing conditions has slowed the speed of these investigations.

In this study, the physical and chemical properties of the sake produced from two different rice varieties were examined during fermentation.

Saccharomyces cerevisiae sake yeast is different than brewery yeasts or wine yeast because of their requirement for pantothenic acid and can tolerate a wide range of growth temperatures and the presence of higher concentration of alcohol (Yoshizawa, 1999). Moto is very important for moromi in sake brew as the yeast starter. Yeast starters divided into two as Kimoto and Yamahaimoto and are acidified by lactic acid-forming bacteria (Yoshizawa, 1999).

Yeast is indispensable for sake brewing though it is not considered a main ingredient. Many kinds of yeast exist in the natural world. The yeast used to brew sake yeast (seishu-kobo), mostly *Saccharomyces cerevisiae*. Different characteristics of various yeasts are strong contributors in deciding the flavor, aroma and quality of sake.

Strains of yeasts with especially high brewing quality are usually used. In many cases, a single strain is not necessarily used alone. The advantages of each strain are combined by mixing several strains or blending the brewed sake. In the wine production, the aroma of grapes largely contributes to the flavor of wine. On the other hand, rice has neither a characteristic flavor nor a profound effect on the flavor and aroma (Furukawa, 2012, Champagne, 2004). For example, the special yeasts employed in ginjo-shu brewing (ginjo-kobo) happen to produce fruity flavors and aromas like banana or apple during fermentation. No artificial flavoring compounds are added (Furukawa, 2012). Recently, in Japan, yeasts taken from various flowers such as cherry blossoms, roses and pinks (called flower yeasts) were tried for sake brewing in many different locations. The sake brewed with flower yeast present different characteristics according to their flower of origin (Furukawa, 2012).

In the present study, *Saccharomyces cerevisiae* (ATCC® 26422™) Kyokai 7 type yeast were used due to being well known as *Saccharomyces sake*.

3.3.1 Physical and Chemical Properties of Karacadağ Rice Sake

Karacadağ rice sake was fermented for 34 days and at each two days intervals, pH, titratable acidity (ml), Brix, alcohol content (ABV %) and color as CIE L*, a*, b* and YI values were measured. The results are given in Tables 3.3 and 3.4

Table 3.3. Change in physical and chemical properties of Karacadağ rice sake during fermentation

	Karacadağ							
Day	pH		Titratable Acidity (ml)		Brix		Alcohol Content (ABV %)	
0	3.94^{k}	(±0.04)	1.65^{a}	(±0.07)	4.32^{a}	(±0.01)	0.00^{a}	(±0.00)
2	3.87^{j}	(±0.01)	3.70^{gh}	(±0.14)	4.47^{ab}	(±0.13)	0.65^{b}	(±0.07)
4	3.53^{a}	(±0.03)	3.30^{efg}	(±0.14)	4.51^{ab}	(±0.37)	1.50^{c}	(±0.00)
6	3.80^{i}	(±0.01)	4.05^{h}	(±0.21)	4.54^{abc}	(±0.01)	2.20^{d}	(±0.00)
8	3.80^{i}	(±0.01)	2.45^{b}	(±0.21)	4.59^{abc}	(±0.23)	3.25^{e}	(±0.07)
10	3.55^{ab}	(±0.01)	2.65^{bcd}	(±0.07)	4.93^{bcd}	(±0.05)	3.80^{f}	(±0.14)
12	3.59^{bc}	(±0.04)	3.05^{cdef}	(±0.21)	4.98^{cde}	(±0.39)	4.70^{g}	(±0.14)
14	3.58^{bc}	(±0.01)	2.60^{bc}	(±0.14)	4.88^{bcd}	(±0.54)	5.70^{h}	(±0.28)
16	3.67^{fgh}	(±0.01)	2.75^{bcde}	(±0.07)	5.29^{def}	(±0.13)	6.40^{i}	(±0.00)
18	3.59^{bc}	(±0.00)	2.35^{b}	(±0.07)	5.40^{efg}	(±0.07)	7.10^{j}	(±0.14)
20	3.69^{gh}	(±0.02)	2.75^{bcde}	(±0.07)	5.47^{fg}	(±0.07)	7.80^{k}	(±0.14)
22	3.66^{fgh}	(±0.01)	3.30^{efg}	(±0.14)	5.52^{fg}	(±0.04)	8.70^{l}	(±0.00)
24	3.66^{fgh}	(±0.02)	3.10^{def}	(±0.14)	6.01^{h}	(±0.13)	9.85^{m}	(±0.07)
26	3.63^{def}	(±0.01)	3.70^{gh}	(±0.14)	5.79^{gh}	(±0.07)	10.20^{n}	(±0.14)
28	3.64^{efg}	(±0.03)	2.55^{b}	(±0.64)	6.02^{h}	(±0.11)	11.05^{o}	(±0.07)
30	3.68^{fgh}	(±0.01)	3.10^{def}	(±0.14)	6.20^{h}	(±0.01)	11.80^{p}	(±0.14)
32	3.61^{cde}	(±0.01)	3.15^{ef}	(±0.07)	6.05^{h}	(±0.04)	12.40^{r}	(±0.00)
34	3.67^{fgh}	(±0.00)	3.25^{efg}	(±0.07)	6.13^{h}	(±0.06)	12.65^{s}	(±0.07)

a, b, c etc. show Duncan Test homogeneous groups (P≤0.05).

The statistical analysis results for pH, titratable acidity, Brix and Alcohol content are given at appendices; A.1 and A.2. Duncan test result for Karacadağ type rice sake is given at appendices A.3, A.5, A.7 and A.8.

Table 3.4. Change in color of Karacadağ rice sake during fermentation

	Karacadağ							
Day	L*		a*		b*		YI	
0	54.67^{bcd}	(±1.12)	-2.57^{abcde}	(±0.13)	-0.16^{abcd}	(±0.57)	-4.05^{abcde}	(±1.39)
2	58.14f^{gh}	(±0.49)	-2.59^{abcd}	(±0.07)	2.22^{f}	(±0.16)	2.66^{g}	(±0.33)
4	53.55^{abcd}	(±1.83)	-2.51^{bcdef}	(±0.06)	-0.44^{abc}	(±1.10)	-5.06^{abcd}	(±3.39)
6	54.51^{bcd}	(±0.01)	-2.63^{ab}	(±0.03)	-0.92^{ab}	(±0.07)	-6.66^{abc}	(±0.04)
8	51.90^{ab}	(±0.35)	-2.45^{def}	(±0.05)	-1.29^{a}	(±0.14)	-7.73^{ab}	(±0.51)
10	59.04^{gh}	(±0.91)	-2.60^{abcd}	(±0.00)	2.48^{f}	(±0.47)	3.31^{g}	(±1.19)
12	55.28^{cdef}	(±1.85)	-2.41^{ef}	(±0.01)	0.52^{bcde}	(±1.03)	-1.94^{def}	(±3.09)
14	55.62^{cdef}	(±0.77)	-2.51^{bcdef}	(±0.02)	0.42^{bcde}	(±0.44)	-2.32^{cdef}	(±1.32)
16	54.09^{bcd}	(±0.01)	-2.46^{cdef}	(±0.01)	0.05^{abcd}	(±0.02)	-3.43^{bcdef}	(±0.08)
18	54.62^{bcde}	(±0.98)	-2.46^{cdef}	(±0.00)	0.37^{bcde}	(±0.57)	-2.42^{cdef}	(±1.73)
20	54.51^{bcd}	(±1.31)	-2.57^{abcde}	(±0.04)	-0.11^{abcd}	(±0.73)	-4.00^{abcde}	(±2.20)
22	54.02^{bcd}	(±0.05)	-2.46^{cdef}	(±0.04)	0.25^{bcde}	(±0.07)	-2.78^{cdef}	(±0.18)
24	50.91^{a}	(±4.17)	-2.39^{f}	(±0.20)	-1.37^{a}	(±1.44)	-8.14^{a}	(±4.86)
26	56.66^{defg}	(±0.70)	-2.66^{ab}	(±0.04)	1.26^{def}	(±0.45)	-0.21^{efg}	(±1.11)
28	57.72^{efgh}	(±0.86)	-2.58^{abcd}	(±0.00)	2.23^{f}	(±0.46)	2.70^{g}	(±1.24)
30	59.92^{h}	(±0.69)	-2.68^{a}	(±0.01)	1.67^{ef}	(±0.58)	1.00^{fg}	(±1.49)
32	52.47^{abc}	(±0.10)	-2.64^{ab}	(±0.01)	-0.81^{abc}	(±0.10)	-6.35^{abcd}	(±0.32)
34	58.22^{fgh}	(±0.75)	-2.61^{abc}	(±0.05)	0.57^{cde}	(±0.04)	-3.60^{bcde}	(±0.54)

a, b, c etc. show Duncan Test homogeneous groups (P≤0.05).

According to the statistical analysis, pH, acidity, Brix, alcohol content, L*, a*, b* and YI values of Karacadağ rice sake significantly (P≤0.05) changed during the fermentation.

The statistical analysis results for Karacadağ rice sake for color values are given at appendices A.10, A.11, A.12, A.14, A.16 and A.18.

3.3.2 Physical and Chemical Properties of Krasnodar Rice Sake

Krasnodar rice sake was fermented for 34 days and each two days intervals pH, acidity (ml), Brix, alcohol content (%) and color as CIE L*, a*b*, YI* were measured. The results are given in Tables 3.5 and 3.6.

Table 3.5. Change in physical and chemical properties of Krasnodar rice sake during fermentation

	Krasnador							
Day	pH		Titratable Acidity (ml)		Brix		Alcohol Content (ABV %)	
0	3.98^{a}	(±0.02)	2.26^{a}	(±0.06)	8.25	(±0.07)	0.00^{a}	(±0.00)
2	4.78^{k}	(±0.01)	3.35^{b}	(±0.07)	7.81	(±0.03)	0.71^{b}	(±0.01)
4	4.51^{j}	(±0.01)	4.60^{fg}	(±0.14)	7.79	(±0.19)	1.89^{c}	(±0.02)
6	$4.40d^{e}$	(±0.01)	4.35^{def}	(±0.07)	7.66	(±0.04)	2.54^{d}	(±0.00)
8	4.35^{bcde}	(±0.01)	5.05^{h}	(±0.21)	7.76	(±0.01)	3.66^{e}	(±0.01)
10	4.36^{cde}	(±0.01)	4.75^{g}	(±0.07)	7.74	(±0.02)	4.71^{f}	(±0.01)
12	4.41^{de}	(±0.01)	5.10^{h}	(±0.14)	8.04	(±0.01)	5.48^{g}	(±0.01)
14	4.29^{bc}	(±0.02)	6.15^{j}	(±0.07)	7.64	(±0.02)	6.30^{h}	(±0.00)
16	4.27^{bc}	(±0.01)	5.50^{i}	(±0.28)	8.15	(±0.07)	6.91^{i}	(±0.01)
18	4.35^{bcde}	(±0.05)	4.05^{c}	(±0.21)	-	-	7.17^{j}	(±0.01)
20	4.25^{b}	(±0.01)	4.10^{cd}	(±0.14)	-	-	7.21^{k}	(±0.00)
22	4.32^{bcd}	(±0.03)	6.65^{k}	(±0.07)	-	-	8.71^{l}	(±0.01)
24	4.44^{efgh}	(±0.05)	4.40^{ef}	(±0.00)	-	-	8.80^{m}	(±0.00)
26	4.43^{efgh}	(±0.01)	4.15^{cde}	(±0.07)	-	-	9.50^{n}	(±0.01)
28	4.50^{ghi}	(±0.00)	3.90^{c}	(±0.14)	-	-	10.23^{o}	(±0.00)
30	4.54^{i}	(±0.16)	2.30^{a}	(±0.14)	-	-	10.59^{p}	(±0.01)
32	4.69^{j}	(±0.02)	3.25^{b}	(±0.07)	-	-	12.27^{r}	(±0.01)
34	4.64^{j}	(±0.01)	3.35^{b}	(±0.07)	-	-	13.80^{s}	(±0.00)

a, b, c etc. show Duncan Test homogeneous groups (P≤0.05).

Note: After 16^{th} day, the brix values were unlogical values. The reason was not predicted.

The statistical analysis results for pH, titratable acidity, Brix and Alcohol content are given at appendices; A.1 and A.2. Duncan test result for Krasnodar type rice sake is given at appendices A.4, A.6 and A.9.

Table 3.6. Change in color of Krasnodar rice sake during fermentation

	Krasnador							
Day	L*		a*		b*		YI	
0	43.36^{a}	(±1.29)	-2.96^{ef}	(±0.03)	1.94^{a}	(±0.06)	1.21^{a}	(±0.05)
2	47.31^{b}	(±1.20)	-3.06^{de}	(±0.01)	2.24^{a}	(±0.62)	2.51^{a}	(±1.94)
4	51.85^{c}	(±1.58)	-3.01^{ef}	(±0.00)	2.63^{a}	(±0.76)	3.56^{a}	(±2.17)
6	59.08^{de}	(±0.11)	-3.43^{bc}	(±0.01)	7.03^{b}	(±0.33)	14.15^{bc}	(±0.81)
8	62.32^{g}	(±0.06)	-3.49^{b}	(±0.06)	8.93^{d}	(±0.29)	18.00^{d}	(±0.61)
10	60.23^{df}	(±1.15)	-3.25^{cd}	(±0.05)	5.94^{b}	(±0.70)	11.41^{b}	(±1.53)
12	72.26^{ij}	(±0.29)	-2.83^{fg}	(±0.14)	14.42^{g}	(±0.17)	27.90^{g}	(±0.40)
14	69.59^{h}	(±0.64)	-3.24^{cd}	(±0.05)	10.78^{e}	(±0.30)	20.79^{e}	(±0.40)
16	57.65^{de}	(±0.19)	-3.78^{a}	(±0.21)	7.25^{bc}	(±1.59)	14.36^{c}	(±3.72)
18	64.89^{g}	(±0.80)	-3.92^{a}	(±0.02)	11.81^{ef}	(±0.17)	23.26^{ef}	(±0.16)
20	66.83^{g}	(±1.32)	-3.74^{a}	(±0.13)	12.36^{f}	(±0.77)	24.09^{ef}	(±1.44)
22	70.76^{hi}	(±0.04)	-3.27^{cd}	(±0.02)	14.75^{gh}	(±0.40)	28.43^{g}	(±0.79)
24	72.64^{ij}	(±0.09)	-2.74^{gh}	(±0.10)	15.83^{hi}	(±0.16)	30.61^{gh}	(±0.31)
26	73.53^{j}	(±0.02)	-2.42^{ij}	(±0.04)	16.84^{i}	(±0.04)	32.49^{h}	(±0.07)
28	72.85^{ij}	(±0.12)	-2.40^{j}	(±0.15)	16.88^{i}	(±0.34)	32.79^{h}	(±0.74)
30	67.09^{g}	(±3.73)	-2.55^{hij}	(±0.08)	15.87^{hi}	(±0.29)	31.71^{h}	(±0.71)
32	72.11^{hij}	(±0.33)	-2.50^{ij}	(±0.10)	16.97^{i}	(±0.11)	32.90^{h}	(±1.10)
34	71.72^{hij}	(±0.09)	-2.61^{hi}	(±0.09)	16.83^{i}	(±0.13)	32.79^{h}	(±0.11)

a, b, c etc. show Duncan Test homogeneous groups (P≤0.05).

According to the statistical analysis, pH, acidity, Brix, alcohol content, L*, a*, b* and YI values of Krasnodar rice sake significantly (P≤0.05) changed during the fermentation.

The statistical analysis results for Karacadağ rice sake for color values are given at appendices A.10, A.11, A.13, A.15, A.17 and A.19.

3.4 Comparison of Karacadağ and Krasnodar Rice Sake

The sake obtained from Karacadağ and Krasnodar were compared based on pH, acidity, Brix and alcohol content (Figures 3.1-3.4). According to Figure 3.1, the pH of Krasnodar sake was higher than Karacadağ one at the start and also during the fermentation. The pH value of Karacadağ sake significantly (P≤0.05) decreased. The pH value of Krasnodar sake significantly (P≤0.05) decreased during 20 days of the

fermentation and then it started to increase (P≤0.05). According to Figure 3.2, the acidity of Krasnodar sake was higher than Karacadağ one at the start and additionally during the fermentation. The acidity values of both Karacadağ and Krasnodar sake significantly (P≤0.05) increased. According to Figure 3.3, the Brix of Krasnodar sake was higher than Karacadağ sake at the start. During the parallel fermentation, sugar is converted to glucose by koji and glucose is used by yeasts cells. The Brix value increases on the one hand while it is used by the yeasts on the other hand. This prevents the rapid increase. As an interesting result, for Krasnodar sake Brix value could not be measured after the day of 16. This can be concluded that the sugar produced in Karacadağ type rice sake vessel consumed more quickly than that of Krasnodar type rice sake vessels. But, a clean explanation was not found. According to Figure 3.4, the alcohol contents of both Krasnodar and Karacadağ sakes were nearly the same at the start and also during the fermentation. The alcohol content of both type significantly (P≤0.05) increased.

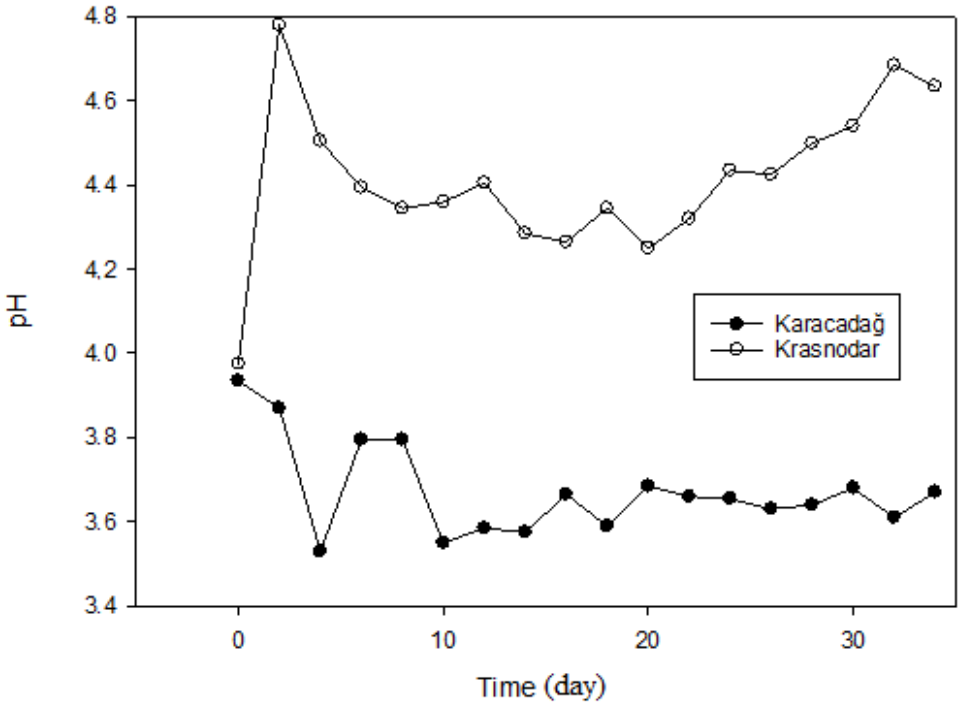

Figure 3.1. pH change during fermentation

The expected final pH for sake is between 4.2 and 4.7 (Japan Sake and Shochu Makers Association and National Research Institute of Brewing, 2011). It was obtained that Krasnodar type sake pH started from 3.98 with fermentation and increased up to 4.69, which was quite suitable for the fermented sake.

The acidity in sake strengthens the taste and hides the sweetness. Sake taste is directly related to acidity. The results show that the acidity starting from low values and then finally reached to the 0.02 (g /100 ml) tartaric acid for both rice types.

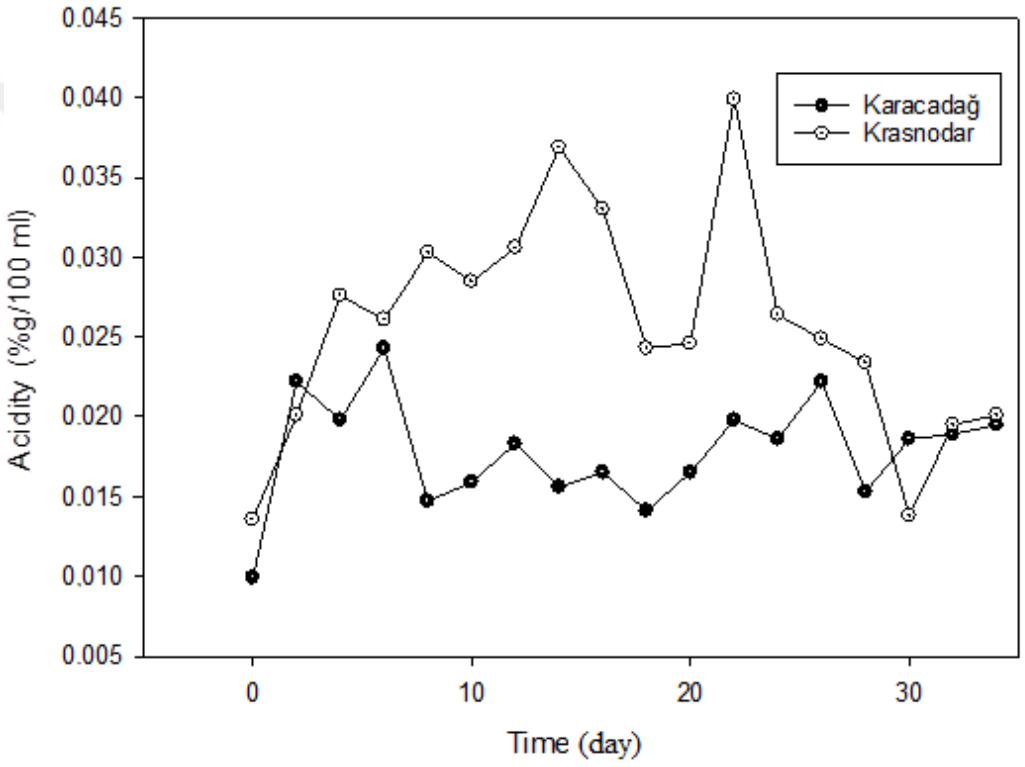

Figure 3.2. Acidity change during fermentation

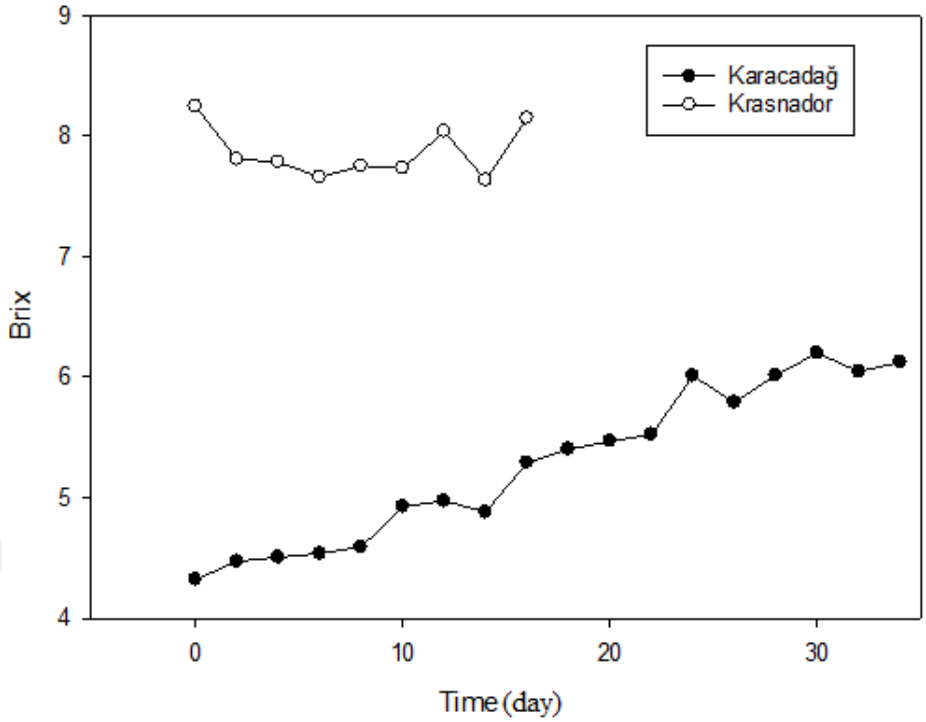

Figure 3.3. Brix change during fermentation

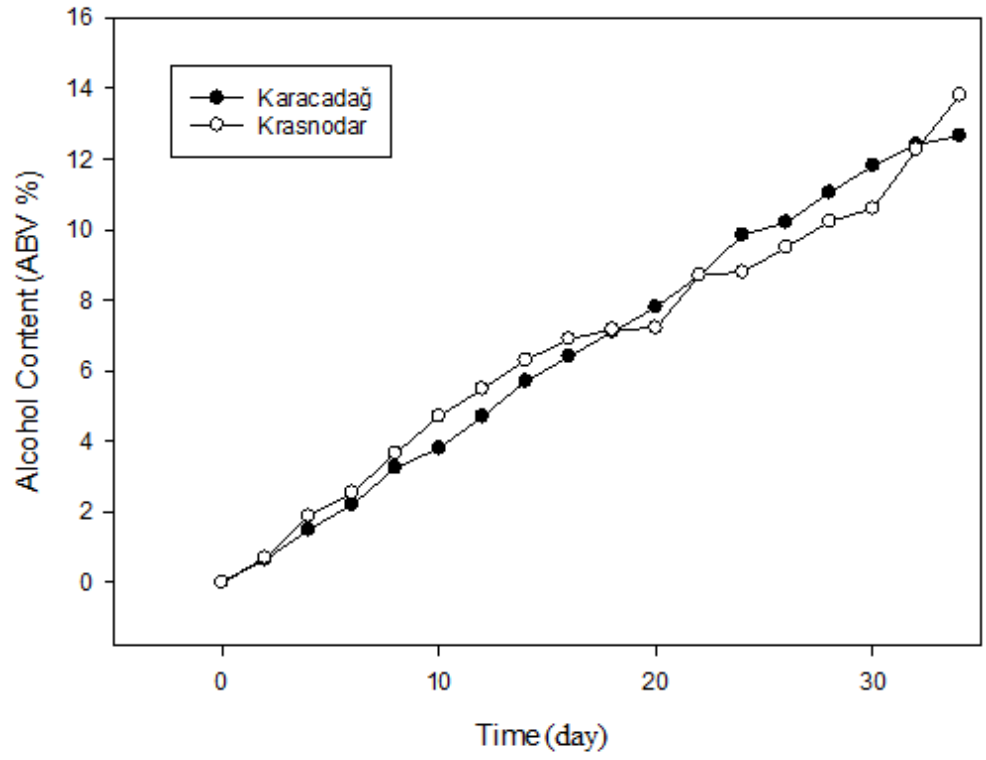

Figure 3.4. Alcohol change during fermentation

Alcohol emerges naturally as a result of fermentation. The steady increase in the percentage of alcohol is the most important proof that fermentation is quite safety.

In a recent study, a total of 1956 Japanese sake samples were analyzed in terms of alcohol content. The alcohol content of the sake samples were between 15.41 (ABV %) ± 0.76 (Mean ± SD) and 15.94 (ABV %) ± 0.89 (Mean ± SD) according to the degree of rice polishing (Furukawa, 2012). In general, the alcohol content of regular sake can be accepted as 13-17 (ABV %) In the present study, the alcohol content of Karacadağ type rice sake and Krasnodar type rice sake were found 12.65 (ABV %) and 13.80 (ABV %), respectively.

The detailed properties of sake available in market are given in details by Furukawa, 2012. The some of them are Ginjo-shu (special brew), Daiginjo-shu (very special brew), Junmai-Ginjo-shu (pure rice, special brew), Junmai-Daiginjo-shu (pure rice, very special brew), Tokubetsu-Junmai-shu (special pure rice), Honjozo-shu (genuine brew), Tokubetsu-Honjozo-shu (special genuine brew) and ippan-shu (normal sake). Nihonshu-do is not a component of sake but an indicator for evaluation. It means sake meter value (SMV) and is a rough indication of the level of sweetness/dryness (specific gravity of sake) (Furukawa, 2012).

In the literature, there are also significant studies on the yeast of sake. Different species and mutants are analyzed (Katou et al.2009, Yano et al. 2003, Nakayama et al. 2012, Fukuda et al. 1998 Shimizu et al. 2005, Fukuda et al. 1999, Suizu 1996, Suizu et al. 1996, Ohbuchi et al. 1996).

Additionally, there are studies about process control and modelling about the sake processing. In the study of Hanai, Nishida et al, 1995, fuzzy controls were studied for fermentation of sake. Experimental fermentations based on two types of fuzzy control were carried out to control Ginjo moromi fermentation automatically. One fermentation (100 kg total rice) was controlled by a Toji (sake-brewing expert), three (1 run with 10 kg total rice; 2 runs with 100 kg total rice) by fuzzy rules based on framework rules. The concentrations of flavor components had almost the same values in all these modes of control, suggesting that Ginjo sake can be under fuzzy control with almost the same quality as that made under manual control of a Toji.

In the study of Kagami et al (1995), the quality of sake was modeled using a neural network as genetic algorithm. The analysis of sake moromi fermentation using the concept of integrated fermentation temperature was made by Yamane et al (1995).

3.5 Sensory Analysis of Sake

The sensory properties of sake are special and it is rich in flavor and aroma. But, they do not come from raw material of rice, which are coming from the brewing and special operations. Especially, mold and yeast used, traditionally techniques, processing parameters and know-how of sake masters are the major determinators of the flavor and aroma of sake.

The flavor and aroma of sake are created with complex and entangled elements of sweetness, dryness, bitterness, sourness and acerbity, additionally umami and aroma all come together to make-up the whole. As the components of flavor and aroma, ordinary and higher alcohols, esters, organic acids, amino acids and peptides can be counted for sake. The details, amounts and their effects are explained in details in the study of Furukawa (2012).

In the evaluation of flavor of sake, it can be classified as flavorful-sake (kun-shu), light smooth sake (so-shu), rich sake (jun-shu) and aged-sake (juku-suhu) (Furukawa, 2012).

Sake has fewer distinctive aromas but a relatively modest and delicate aroma mainly developed during fermentation. The main flavoring compounds are higher alcohol, isoamyl acetate, ethyl caproate and phenyl ethyl acetate, all of which give a distinctive accent to the sake aroma (Yoshizawa, 1999). Although about 300 components have been identified in sake, it is still not clear, which are main flavor contributors.

For example, some contributors are ethyl acetate (fruity and sweet aroma), ethyl acetate (flowery aroma), isobutyl acetate (flowery aroma), ethyl caproate (delicate aroma), and tyrosol together with some amino acids (astringency, bitterness, thick and rough taste). Sotolon, which is present in aged sake, gives a honey-like, laojiu-like aroma (Yoshizawa, 1999). The mechanism of the aroma and flavor formations is explained by Yoshizawa 1999 and Kanauchi 2013.

In the study, 25 panelists were used in the sensory analysis. Karacadağ (abbreviated as A), Krasnodar (abbreviated as C) rice sakes and commercially produced sake (abbreviated as B) (Hakutsuru Sake Brewing with 15.5 % alcohol content) has been tested in terms of taste, odor and overall acceptance. Panelists had never drunk sake before. ANOVA test were applied to the sensory analysis data. It is shown that Karacadağ rice sake has significantly ($P \leq 0.05$) more fruity odor than the others. In terms of overall odor evaluation Karacadağ rice sake significantly ($P \leq 0.05$) more acceptable than the others. According to sensory analysis taste data commercial sake significantly ($P \leq 0.05$) has more mouth feel than the others. On the other hand, Karacadağ rice sake significantly ($P \leq 0.05$) has more fullness taste than the others.

The sensory analysis whole data are given at appendix A.23 and the sensory analysis statistical results are given at appendices A.20 and A.21.

The sensory analysis scale was synthesized from the study of Kanauchi (2013). In that publication, a flavor wheel of sake is given (Figure 3.5).

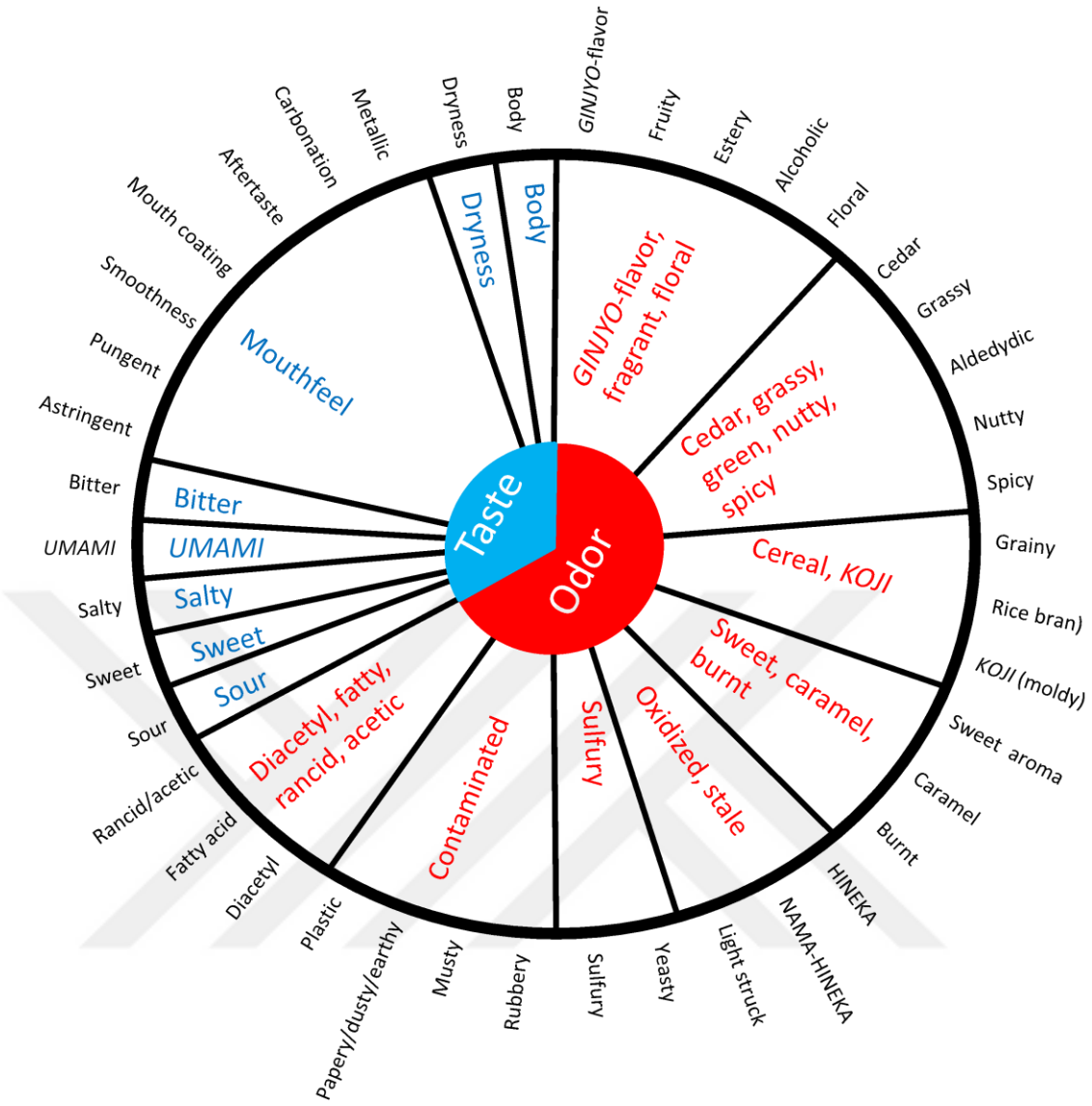

Figure 3.5. Flavor wheel of sake (Kanauchi, 2013)

In the literature, there are a lot of studies related to sensory properties and analysis for sake (Maeda et al. 2011, Arikawa et al. 1996, Takahashi et al. 2016, Yavaş & Rapp 1995, Mimura et al. 2014, Matsuura et al. 1995).

Beside of aroma or flavor, the taste of sake is quite delicate and complicated. However, the taste could be adequately represented by three major factors; sweetness (amakara), fullness (kosa) and cleanness (kireisa) (Iiyama, 1996).

As an off-flavor, hiochi bacteria are important in the finished product. Certain enzymes and alcohol-philic and alcohol-resistant lactic acid bacteria known as hiochi bacteria remain in unpasteurized sake after the brewing stage; these bacteria causing

white turbidity, increasing acid content and generating off-flavors (Kobayashi et al.2016).

Another off-flavoring occurs during the storage of sake, which is known as hineka. Di-methyl tri-sulfide was identified as the main component of hineka. This component is volatile sulfur compound with a sulfurous flavor (Sasaki et al. 2014).

3.6 Overall Comparison

The quality of rice used in sake brewing is well known to affect the taste of sake, but its details are not fully explained. In the production of sake, usually Japonica type short rice grains are used. In other Asian countries short grain rice, except for Japonica type, can be used in alcoholic beverage production (Kanauchi 2013). In order to understand the grain size, the weight of 1000 grains is usually taken into account (Kanauchi 2013). The study reveals that Turkish short grain types (Karacadağ and Krasnodar) are also suitable for sake production with the weight of 1,000 kernels of 22.54 (±0.07) and 23.61 (±0.07) respectively.

The outer part of the brown rice contains lots of lipids, minerals and protein. If the rice used in the sake brew have too much of these components, the undesired aroma occurs. Therefore, these components are removed by polishing. Polishing ratio is an important factor in determining the quality of sake. As the polishing rate increases, sake quality increases. Generally, rice having 70 % polishing ratio is used for ordinary sake brewing. Rice used in this study was polished to 70 %. The study reveals that Karacadağ and Krasnodar rice types with 70 % polishing ratio are sufficiently valuable to produce ordinary sake.

Steaming is extremely important because it leads to further processing. Here, starch is provided to the gelatinized. With the steaming process, the protein is denatured, starch is converted to the α-form and moreover the grains are sterilized. Generally rice grains are steamed for 30-60 minutes (Kanauchi, 2013). In this study, it was determined that rice should be steamed at 100-110 min for the gelatinization of starch.

Traditionally, koji is incubated in wooden boxes inside the Koji-muro, a special temperature and moisture controlled room. There are machines with automatic humidity and temperature control to produce commercial koji. Similarly, koji produced in this study is incubated in temperature and humidity controlled oven. Resulting koji was completely similar to the koji described in the literature.

In the literature, yeast starters are grouped according to methods of preparation. Kimoto and Yamahaimoto types are made by adding lactic acid-forming bacteria. However, another type, Sokuzyo, is made by adding lactic acid directly during the preparation. In Kimoto and Yamahaimoto, different microfloras have been transferred, but only sake yeast grows in Sokuzyo. All yeast starter types initially have up to 20% sugar accumulation. Thus, the growth of microbial contaminants is prevented with low pH and temperature and nitrite formation, and the growth of sake yeast is facilitated in the main fermentation (Yoshizawa, 1999). In this study, Sokuzyo type yeast starter were prepared in order to eliminate the risk of the contamination.

As mentioned in the literature, steamed rice, water and koji are gradually added to the yeast starter for the main fermentation. At this stage, white rice is added about twice as much as the yeast start. Thus, alcohol concentration and yeast population are diluted to about one-third, but the inhibitory effect against microbial contaminants remains. The temperature of main fermentation is 12°C (Yoshizawa, 1999). In this study, main fermentation was take place at 9 °C.

Two days after additions, the yeast population approaches the initial amount. In the second addition, twice as much rice is added as the first addition. The temperature is held at 9-10°C (Yoshizawa, 1999). The next day, the third addition of materials is made by adding the remaining batch of rice at 7-8°C. The rate of fermentation and yeast growth gradually increases, and by the 6^{th}-9^{th} day, the temperature reaches to 10-17°C. This temperature is maintained several days and falls on the following days with the ripening of fermentation. The fermentation is monitored between 15^{th} -30^{th} days, the fermentation is about to end when the alcohol concentration reaches 17-19%. At this stage, a small amount of alcohol can be added to prevent contamination by lactic acid bacteria during storage and to alleviate sake taste.

Thus, the alcohol concentration in the main mash increases to 20-22%. In the final stage, usually a small amount of steamed rice is added. This helps to sweeten the taste (Yoshizawa, 1999). In this study, no alcohol is added into the main fermentation vessel to observe naturally occurring alcohol content and the aroma.

As a result, sake made from Turkish rice types taking into account the critical points mentioned in the literature is close to the quality and the sensory properties of the Japan sakes in terms of both alcohol amount and aroma values.

3.7 Conclusions

In the present study the following conclusions were derived:

- The preferred 70% polishing ratio for both rice varieties is sufficient to obtain sake of the desired quality. A further reduction in the polishing ratio will not be economical.
- Emery stone type polisher with 0.8 mm jacket screen is sufficient to polishing operation.
- As mentioned in the literature for the production of Koji, private rooms are not essential; an oven with a moisture content control is sufficient to incubate the koji.
- The short rice varieties produced in Turkey were detected suitable for sake brewing process.
- Cooking time was detected as 100-110 min. for the Turkish short type rice varieties.
- Without fermentation, open fermentation continued for about 34 days without contamination. Open fermentation is a process that should be examined alone.
- The protein content of polished Karacadağ and Krasnodar type rice grains was 5.11 (±0.11) % (d.b) and 6.33 (±0.05) % (d.b), respectively. In this case, sake made from Karacadağ rice can be expected to be more acceptable.
- Karacadağ rice sake is significantly more close to the original sake in terms of sensory profile ($P>0.05$).

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APPENDIX

A1. Homogeneity Test for Fermentation Parameters

Table A.1. Homogeneity Test for Fermentation Parameters

		Sum of Squares	df	Mean Square	F	Sig.
pH Karacadağ	Between Groups	.409	17	.024	67.732	0.000
	Within Groups	.006	18	0.000		
	Total	.416	35			
pH Krasnodar	Between Groups	1.137	17	.067	37.389	0.000
	Within Groups	.032	18	.002		
	Total	1.169	35			
Acidity Karacadağ	Between Groups	11.021	17	.648	16.321	0.000
	Within Groups	.715	18	.040		
	Total	11.736	35			
Acidity Krasnodar	Between Groups	46.491	17	2.735	157.170	0.000
	Within Groups	.313	18	.017		
	Total	46.804	35			
Brix Karacadağ	Between Groups	14.351	17	.844	21.206	0.000

	Within Groups	.717	18	.040		
	Total	15.068	35			
Brix Krasnodar	Between Groups	.787	8	.098	17.627	0.000
	Within Groups	.050	9	.006		
	Total	.837	17			
Alcohol Karacadağ	Between Groups	589.025	17	34.649	2771.881	0.000
	Within Groups	.225	18	.013		
	Total	589.250	35			
Alcohol Krasnodar	Between Groups	523.827	17	30.813	396171.496	0.000
	Within Groups	.001	18	0.000		
	Total	523.828	35			

A2. ANOVA Results for Fermentation Parameters

Table A.2. Anova Results for Fermentation Parameters

		Sum of Squares	df	Mean Square	F	Sig.
pH Karacadağ	Between Groups	.409	17	.024	67.732	.000
	Within Groups	.006	18	.000		
	Total	.416	35			
pH Krasnodar	Between Groups	1.137	17	,067	37,389	,000
	Within Groups	.032	18	.002		
	Total	1.169	35			
Acidity Karacadağ	Between Groups	11.021	17	.648	16.321	.000
	Within Groups	.715	18	.040		
	Total	11.736	35			
Acidity Krasnodar	Between Groups	46.491	17	2.735	157.170	.000
	Within Groups	.313	18	.017		
	Total	46.804	35			
Brix Karacadağ	Between Groups	14.351	17	.844	21.206	.000
	Within Groups	.717	18	.040		
	Total	15.068	35			
Brix Krasnodar	Between Groups	.787	8	.098	17.627	.000
	Within Groups	.050	9	.006		

	Total	.837	17			
Alcohol Karacadağ	Between Groups	589.025	17	34.649	2771.881	.000
	Within Groups	.225	18	.013		
	Total	589.250	35			
Alcohol Krasnodar	Between Groups	523.827	17	30.813	396171.496	.000
	Within Groups	.001	18	.000		
	Total	523.828	35			

A3. Duncan Test Results pH Karacadağ

Table A.3. Duncan Test Results pH Karacadağ

		Subset for alpha = 0.05										
Day	N	1	2	3	4	5	6	7	8	9	10	11
4	2	3.5300										
10	2	3.5500	3.5500									
14	2		3.5750	3.5750								
12	2		3.5850	3.5850								
18	2		3.5900	3.5900	3.5900							
32	2			3.6100	3.6100	3.6100						
26	2				3.6300	3.6300	3.6300					
28	2					3.6400	3.6400	3.6400				
24	2						3.6550	3.6550	3.6550			
22	2						3.6600	3.6600	3.6600			
16	2						3.6650	3.6650	3.6650			
34	2						3.6700	3.6700	3.6700			
30	2							3.6800	3.6800			
20	2								3.6850			
6	2									3.7950		
8	2									3.7950		
2	2										3.8700	
0	2											3.9350
Sig.		,303	,066	,104	,058	,148	,074	,074	,173	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2,000.

A4. Duncan Test Results pH Krasnodar

Table A.4. Duncan Test Results pH Krasnodar

		Subset for alpha = 0.05									
Day	N	1	2	3	4	5	6	7	8	9	10
0	2	3.9750									
20	2		4.2500								
16	2		4.2650	4.2650							
14	2		4.2850	4.2850							
22	2		4.3200	4.3200	4.3200						
8	2		4.3450	4.3450	4.3450	4.3450					
18	2		4.3450	4.3450	4.3450	4.3450					
10	2			4.3600	4.3600	4.3600					
6	2				4.3950	4.3950					
12	2				4.4050	4.4050	4.4050				
26	2					4.4250	4.4250	4.4250			
24	2					4.4350	4.4350	4.4350			
28	2						4.5000	4.5000	4.5000		
4	2							4.5050	4.5050		
30	2								4.5400		
34	2									4.6350	
32	2									4.6850	
2	2										4.7800
Sig.		1.000	.060	.060	.090	.076	.052	.098	.383	.253	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

A5. Duncan Test Results Acidity Karacadağ

Table A.5. Duncan Test Results Acidity Karacadağ

		Subset for alpha = 0.05							
Day	N	1	2	3	4	5	6	7	8
0	2	1.6500							
18	2		2.3500						
8	2		2.4500						
28	2		2.5500						
14	2		2.6000	2.6000					
10	2		2.6500	2.6500	2.6500				
16	2		2.7500	2.7500	2.7500	2.7500			
20	2		2.7500	2.7500	2.7500	2.7500			
12	2			3.0500	3.0500	3.0500	3.0500		
24	2				3.1000	3.1000	3.1000		
30	2				3.1000	3.1000	3.1000		
32	2					3.1500	3.1500		
34	2						3.2500	3.2500	
4	2						3.3000	3.3000	
22	2						3.3000	3.3000	
2	2							3.7000	3.7000
26	2							3.7000	3.7000
6	2								4.0500
Sig.		1.000	.093	.055	.059	.090	.282	.055	.113

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

A6. Duncan Test Results Acidity Krasnodar

Table A.6. Duncan Test Results Acidity Krasnodar

		Subset for alpha = 0.05										
Day	N	1	2	3	4	5	6	7	8	9	10	11
0	2	2.2600										
30	2	2.3000										
32	2		3.2500									
2	2		3.3500									
34	2		3.3500									
28	2			3.9000								
18	2			4.0500								
20	2			4.1000	4.1000							
26	2			4.1500	4.1500	4.1500						
6	2				4.3500	4.3500	4.3500					
24	2					4.4000	4.4000					
4	2						4.6000	4.6000				
10	2							4.7500				
8	2								5.0500			
12	2								5.1000			
16	2									5.5000		
14	2										6.1500	
22	2											6.6500
Sig.		.765	.483	.097	.088	.088	.088	.270	.709	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

A7. Duncan Test Results Brix Karacadağ

Table A.7. Duncan Test Results Brix Karacadağ

		Subset for alpha = 0.05							
Day	N	1	2	3	4	5	6	7	8
0	2	4.3200							
2	2	4.4700	4.4700						
4	2	4.5050	4.5050						
6	2	4.5350	4.5350	4.5350					
8	2	4,5900	4,5900	4,5900					
14	2		4,8800	4,8800	4,8800				
10	2		4,9250	4,9250	4,9250				
12	2			4,9750	4,9750	4,9750			
16	2				5,2900	5,2900	5,2900		
18	2					5,4000	5,4000	5,4000	
20	2						5,4700	5,4700	
22	2						5,5200	5,5200	
26	2							5,7900	5,7900
24	2								6,0100
28	2								6,0150
32	2								6,0450
34	2								6,1250
30	2								6.2000
Sig.		.238	.056	.061	.074	.058	.304	.088	.083

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

A8. Duncan Test Results Alcohol Karacadağ

Table A.8. Duncan Test Results Alcohol Karacadağ

		Subset for alpha = 0.05																	
Day	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	2	.0000																	
2	2		.6500																
4	2			1.5000															
6	2				2.2000														
8	2					3.2500													
10	2						3.8000												
12	2							4.7000											
14	2								5.7000										
16	2									6.4000									
18	2										7.1000								
20	2											7.8000							
22	2												8.7000						
24	2													9.8500					
26	2														10.2000				
28	2															11.0500			
30	2																11.8000		
32	2																	12.4000	
34	2																		12.6500
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

A9. Duncan Test Results Alcohol Krasnodar

Table A.9. Duncan Test Results Alcohol Krasnodar

		Subset for alpha = 0.05																
Day	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	2	.0000																	
2	2		.7050																
4	2			1.8850															
6	2				2.5400														
8	2					3.6600													
10	2						4.7100												
12	2							5.4750											
14	2								6.3000										
16	2									6.9050									
18	2										7.1650								
20	2											7.2100							
22	2												8.7050						
24	2													8.8000					
26	2														9.4950				
28	2															10.2300			
30	2																10.5900		
32	2																	12.2650	
34	2																		13.8000
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

A10. Homogeneity Test for Color Change during Fermentation

Table A.10. Homogeneity Test for Color Change during Fermentation

		Levene Statistic	df1	df2	Sig.
L* Karacadag					
	Based on Mean	8.498E+28	17	18	.000
	Based on Median	8.498E+28	17	18	.000
	Based on Median and with adjusted df	8,498E+28	17	8,000	,000
	Based on trimmed mean	1,545E+28	17	18	,000
L* Krasnodar					
	Based on Mean	2.015E+28	17	18	.000
	Based on Median	2.015E+28	17	18	.000
	Based on Median and with adjusted df	2.015E+28	17	8.824	.000
	Based on trimmed mean	6.432E+27	17	18	.000
a* Karacadag					
	Based on Mean	4.751E+28	17	18	.000
	Based on Median	4.751E+28	17	18	.000

	Based on Median and with adjusted df	4.751E+28	17	10.000	.000
	Based on trimmed mean	1.827E+28	17	18	.000
a* Krasnodar					
	Based on Mean	7.475E+28	17	18	.000
	Based on Median	7.475E+28	17	18	.000
	Based on Median and with adjusted df	7.475E+28	17	8.000	.000
	Based on trimmed mean	2.136E+28	17	18	.000
b* Karacadag					
	Based on Mean	8.938E+30	17	18	.000
	Based on Median	8.938E+30	17	18	.000
	Based on Median and with adjusted df	8.938E+30	17	4.652	.000
	Based on trimmed mean	2.302E+30	17	18	.000
b* Krasnodar	Based on Mean	1.109E+29	17	18	.000
	Based on Median	1.109E+29	17	18	.000

	Based on Median and with adjusted df	1.109E+29	17	4.344	.000
	Based on trimmed mean	6.089E+28	17	18	.000
YI* Karacadag					
	Based on Mean	1.181E+31	17	18	.000
	Based on Median	1.181E+31	17	18	.000
	Based on Median and with adjusted df	1.181E+31	17	7.918	.000
	Based on trimmed mean	4.715E+30	17	18	.000
YI* Krasnodar					
	Based on Mean	1.787E+29	17	18	.000
	Based on Median	1.787E+29	17	18	.000
	Based on Median and with adjusted df	1.787E+29	17	3.931	.000
	Based on trimmed mean	6.389E+28	17	18	.000

A11. ANOVA Results for Color Change during Fermentation

Table A.11. ANOVA Results for Colour Change during Fermentation

		Sum of Squares	df	Mean Square	F	Sig.
L* Karacadag	Between Groups	213.604	17	12.565	7.031	.000
	Within Groups	32.168	18	1.787		
	Total	245.772	35			
L* Krasnodar	Between Groups	2930.609	17	172.389	130.161	.000
	Within Groups	23,840	18	1,324		
	Total	2954,448	35			
a* Karacadag	Between Groups	,267	17	,016	3,663	,005
	Within Groups	,077	18	,004		
	Total	,344	35			
a*Krasnodar	Between Groups	7,864	17	,463	56,373	,000
	Within Groups	,148	18	,008		
	Total	8,011	35			
b* Karacadag	Between Groups	47,874	17	2,816	7,524	,000
	Within Groups	6,737	18	,374		
	Total	54,611	35			
b* Krasnodar	Between Groups	1004.430	17	59.084	199.322	.000
	Within Groups	5.336	18	.296		
	Total	1009.765	35			
YI* Karacadag	Between Groups	417.574	17	24.563	6.984	.000
	Within Groups	63.308	18	3.517		
	Total	480.882	35			
YI* Krasnodar	Between Groups	4209.338	17	247.608	143.234	.000
	Within Groups	31.117	18	1.729		
	Total	4240.454	35			

A12. Duncan Test Results L* Karacadağ

Table A.12. Duncan Test Results L* Karacadağ

		Subset for alpha = 0.05							
Day	N	1	2	3	4	5	6	7	8
24	2	50.9100a							
8	2	51.9000ab	51.9000ab						
32	2	52.4700abc	52.4700abc	52.4700abc					
4	2	53.5450abcd	53.5450abcd	53.5450abcd	53.5450abcd				
22	2		54.0150bcd	54.0150bcd	54.0150bcd				
16	2		54.0850bcd	54.0850bcd	54.0850bcd				
6	2		54.5050bcd	54.5050bcd	54.5050bcd				
20	2		54.5050bcd	54.5050bcd	54.5050bcd				
18	2		54.6200bcde	54.6200bcde	54.6200bcde	54.6200bcde			
0	2		54.6650bcde	54.6650bcde	54.6650bcde	54.6650bcde			
12	2			55.2750cdef	55.2750cdef	55.2750cdef	55.2750cdef		
14	2			55.6150cdef	55.6150cdef	55.6150cdef	55.6150cdef		
26	2				56.6550defg	56.6550defg	56.6550defg	56.6550defg	
28	2					57.7200efgh	57.7200efgh	57.7200efgh	57.7200efgh
2	2						58.1400fgh	58.1400fgh	58.1400fgh
34	2						58.2200fgh	58.2200fgh	58.2200fgh
10	2							59.0350gh	59.0350gh
30	2								59.9150h
Sig.		.086	.088	.056	.058	.053	.065	.125	.156

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A13. Duncan Test Results L* Krasnodar

Table A.13. Duncan Test Results L* Krasnodar

		Subset for alpha = 0.05									
Day	N	1	2	3	4	5	6	7	8	9	10
0	2	43.3600a									
2	2		47.3050b								
4	2			51.8500c							
16	2				57.6450d						
6	2				59.0750de	59.0750de					
10	2					60.2300df	60.2300df				
8	2						62.3150f				
18	2							64.8850g			
20	2							66.8300g			
30	2							67.0850g			
14	2								69.5900h		
22	2								70.7550hi	70.7550hi	
34	2								71.7150hij	71.7150hij	71.7150hij
32	2								72.1100hij	72.1100hij	72.1100hij
12	2									72.2550ij	72.2550ij
24	2									72.6350ij	72.6350ij
28	2									72.8450ij	72.8450ij
26	2										73.5250j
Sig.		10.000	10.000	10.000	.230	.329	.087	.086	.058	.123	.178

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A14. Duncan Test Results a* Karacadağ

Table A.14. Duncan Test Results a* Karacadağ

		Subset for alpha = 0.05					
Day	N	1	2	3	4	5	6
30	2	-2.6750a					
26	2	-2.6550ab	-2.6550ab				
32	2	-2.6400ab	-2.6400ab				
6	2	-2.6300ab	-2.6300ab				
34	2	-2.6050abc	-2.6050abc	-2.6050abc			
10	2	-2.6000abcd	-2.6000abcd	-2.6000abcd	-2.6000abcd		
2	2	-2.5900abcd	-2.5900abcd	-2.5900abcd	-2.5900abcd		
28	2	-2.5800abcd	-2.5800abcd	-2.5800abcd	-2.5800abcd		
0	2	-2.5650abcde	-2.5650abcde	-2.5650abcde	-2.5650abcde	-2.5650abcde	
20	2	-2.5650abcde	-2.5650abcde	-2.5650abcde	-2.5650abcde	-2.5650abcde	
4	2		-2.5050bcdef	-2.5050bcdef	-2.5050bcdef	-2.5050bcdef	-2.5050bcdef
14	2		-2.5050bcdef	-2.5050bcdef	-2.5050bcdef	-2.5050bcdef	-2.5050bcdef
18	2			-2.4600cdef	-2.4600cdef	-2.4600cdef	-2.4600cdef
22	2			-2.4600cdef	-2.4600cdef	-2.4600cdef	-2.4600cdef
16	2			-2.4550cdef	-2.4550cdef	-2.4550cdef	-2.4550cdef
8	2				-2.4450def	-2.4450def	-2.4450def
12	2					-2.4100ef	-2.4100ef
24	2						-2.3900f
Sig.		.161	.063	.063	.056	.054	.141

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A15. Duncan Test Results a* Krasnodar

Table A.15. Duncan Test Results a* Krasnodar

		Subset for alpha = 0.05									
Day	N	1	2	3	4	5	6	7	8	9	10
18	2	-3.9150a									
16	2	-3.7750a									
20	2	-3.7400a									
8	2		-3.4850b								
6	2		-3.4250bc	-3.4250bc							
22	2			-3.2650cd	-3.2650						
10	2			-3.2450cd	-3.2450						
14	2			-3.2350cd	-3.2350						
2	2				-3.0600de	-3.0600de					
4	2					-3.0100ef	-3.0100ef				
0	2					-2.9600ef	-2.9600ef				
12	2						-2.8300fg	-2.8300fg			
24	2							-2.7400gh	-2.7400gh		
34	2								-2.6050hi	-2.6050hi	
30	2								-2.5500hij	-2.5500hij	-2.5500hij
32	2									-2.5000ij	-2.5000ij
26	2									-2.4150ij	-2.4150ij
28	2										-2.3950j
Sig.		.083	.516	.068	.051	.310	.075	.334	.061	.068	.132

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A16. Duncan Test Results b* Karacadağ

Table A.16. Duncan Test Results b* Karacadağ

		Subset for alpha = 0.05					
Day	N	1	2	3	4	5	6
24	2	-1.3650a					
8	2	-1.2900a					
6	2	-.9200ab	-.9200ab				
32	2	-.8100abc	-.8100abc	-.8100abc			
4	2	-.4350abc	-.4350abc	-.4350abc			
0	2	-.1600abcd	-.1600abcd	-.1600abcd	-.1600abcd		
20	2	-.1050abcd	-.1050abcd	-.1050abcd	-.1050abcd		
16	2	.0450abcd	.0450abcd	.0450abcd	.0450abcd		
22	2		.2500bcde	.2500bcde	.2500bcde	.2500bcde	
18	2		.3650bcde	.3650bcde	.3650bcde	.3650bcde	
14	2		.4200bcde	.4200bcde	.4200bcde	.4200bcde	
12	2		.5200bcde	.5200bcde	.5200bcde	.5200bcde	
34	2			.5700cde	.5700cde	.5700cde	
26	2				1.2600def	1.2600def	1.2600def
30	2					1.6700ef	1.6700ef
2	2						2.2150f
28	2						2.2250f
10	2						2.4800f
Sig.		.058	.056	.066	.058	.055	.088

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A17. Duncan Test Results b* Krasnodar

Table A.17. Duncan Test Results b* Krasnodar

		Subset for alpha = 0.05								
Day	N	1	2	3	4	5	6	7	8	9
0	2	1.9400a								
2	2	2.2400a								
4	2	2.6300a								
10	2		5.9350b							
6	2		7.0300b	7.0300bc						
16	2			7.2450bc						
8	2				8.9250d					
14	2					10.7800e				
18	2					11.8100ef	11.8100ef			
20	2						12.3550f			
12	2							14.4200g		
22	2							14.7500gh	14.7500gh	
24	2								15.8300hi	15.8300hi
30	2								15.8650hi	15.8650hi
34	2									16.8300i
26	2									16.8400i
28	2									16.8800i
32	2									16.9700i
Sig.		.246	.060	.698	10.000	.075	.330	.552	.067	.078

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A18. Duncan Test Results YI* Karacadağ

Table A.18. Duncan Test Results YI* Karacadağ

		Subset for alpha = 0.05						
Day	N	1	2	3	4	5	6	7
24	2	-8.1350a						
8	2	-7.7300ab	-7.7300ab					
6	2	-6.6550abc	-6.6550abc	-6.6550abc				
32	2	-6.3450abcd	-6.3450abcd	-6.3450abcd	-6.3450abcd			
4	2	-5.0550abcd	-5.0550abcd	-5.0550abcd	-5.0550abcd			
0	2	-4.0500abcde	-4.0500abcde	-4.0500abcde	-4.0500abcde	-4.0500abcde		
20	2	-3.9950abcde	-3.9950abcde	-3.9950abcde	-3.9950abcde	-3.9950abcde		
34	2		-3.5950bcde	-3.5950bcde	-3.5950bcde	-3.5950bcde		
16	2		-3.4300bcdef	-3.4300bcdef	-3.4300bcdef	-3.4300bcdef	-3.4300bcdef	
22	2			-2.7750cdef	-2.7750cdef	-2.7750cdef	-2.7750cdef	
18	2			-2.4200cdef	-2.4200cdef	-2.4200cdef	-2.4200cdef	
14	2			-2.3200cdef	-2.3200cdef	-2.3200cdef	-2.3200cdef	
12	2				-1.9350def	-1.9350def	-1.9350def	
26	2					-.2050efg	-.2050efg	-.2050efg
30	2						.9950fg	.9950fg
2	2							2.6550g
28	2							2.7000g
10	2							3.3100g
Sig.		.067	.059	.060	.056	.090	.051	.107

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A19. Duncan Test Results YI* Krasnodar

Table A.19. Duncan Test Results YI* Krasnodar

		Subset for alpha = 0.05							
Day	N	1	2	3	4	5	6	7	8
0	2	1.2050a							
2	2	2.5050a							
4	2	3.5550a							
10	2		11.4100b						
6	2		14.1500bc	14.1500bc					
16	2			14.3600c					
8	2				180.0000d				
14	2					20.7850e			
18	2					23.2600ef	23.2600ef		
20	2						24.0900ef		
12	2							27.8950g	
22	2							28.4300g	
24	2							30.6100gh	30.6100gh
30	2								31.7050h
26	2								32.4900h
28	2								32.7850h
34	2								32.7900h
32	2								32.8950h
Sig.		.107	.052	.875	10.000	.076	.536	.065	.139

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 20.000.

A20. Anova Test Results for Sensory Analyses

Table A.20. Anova Test Results for Sensory Analyses

		Sum of Squares	df	Mean Square	F	Sig.
Fruity	Between Groups	8.667	2	4.333	.889	.415
	Within Groups	350.880	72	4.873		
	Total	359.547	74			
Grassy	Between Groups	27.307	2	13.653	4.008	.022
	Within Groups	245.280	72	3.407		
	Total	272.587	74			
Spicy	Between Groups	18.320	2	9.160	2.457	.093
	Within Groups	268.400	72	3.728		
	Total	286.720	74			
Moldy	Between Groups	25.707	2	12.853	3.384	.039
	Within Groups	273.440	72	3.798		
	Total	299.147	74			
Caramel	Between Groups	6.587	2	3.293	.730	.485
	Within Groups	324.800	72	4.511		
	Total	331.387	74			
O.Odor	Between Groups	1.520	2	.760	.162	.851
	Within Groups	338.400	72	4.700		
	Total	339,920	74			
Fullness	Between Groups	1,280	2	,640	,128	,880

	Within Groups	361,040	72	5,014		
	Total	362,320	74			
Alcohol	Between Groups	101.840	2	50.920	11.380	.000
	Within Groups	322.160	72	4.474		
	Total	424.000	74			
Metallic	Between Groups	6.427	2	3.213	.655	.522
	Within Groups	353.120	72	4.904		
	Total	359.547	74			
Sourness	Between Groups	23.387	2	11.693	2.532	.087
	Within Groups	332.560	72	4.619		
	Total	355.947	74			
Sweetness	Between Groups	16.987	2	8.493	1.765	.179
	Within Groups	346.560	72	4.813		
	Total	363.547	74			
Bitterness	Between Groups	5.227	2	2.613	.582	.562
	Within Groups	323.520	72	4.493		
	Total	328.747	74			
MouthFeel	Between Groups	17.627	2	8.813	2.388	.099
	Within Groups	265.760	72	3.691		
	Total	283.387	74			
O.Evaluation	Between Groups	30.107	2	15.053	3.714	.029
	Within Groups	291.840	72	4.053		
	Total	321.947	74			

A21. Homogeneous Test Results for Sensory Analyses

Table A.21. Homogeneous Test Results for Sensory Analyses

Dependent Variable		(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Fruity	Tukey HSD	A	B	.60000	.62439	.604	-.8942	2.0942
			C	.80000	.62439	.410	-.6942	2.2942
		B	A	-.60000	.62439	.604	-2.0942	.8942
			C	.20000	.62439	.945	-1.2942	1.6942
		C	A	-.80000	.62439	.410	-2.2942	.6942
			B	-.20000	.62439	.945	-1.6942	1.2942
Grassy	Tukey HSD	A	B	1.28000*	.52205	.043	.0307	2.5293
			C	.00000	.52205	1.000	-1.2493	1.2493
		B	A	-1.28000*	.52205	.043	-2.5293	-.0307
			C	-1.28000*	.52205	.043	-2.5293	-.0307
		C	A	.00000	.52205	1.000	-1.2493	1.2493
			B	1.28000*	.52205	.043	.0307	2.5293
Spicy	Tukey HSD	A	B	1.16000	.54610	.092	-.1469	2.4669
			C	.28000	.54610	.865	-1.0269	1.5869
		B	A	-1.16000	.54610	.092	-2.4669	.1469
			C	-.88000	.54610	.247	-2.1869	.4269
		C	A	-.28000	.54610	.865	-1.5869	1.0269
			B	.88000	.54610	.247	-.4269	2.1869

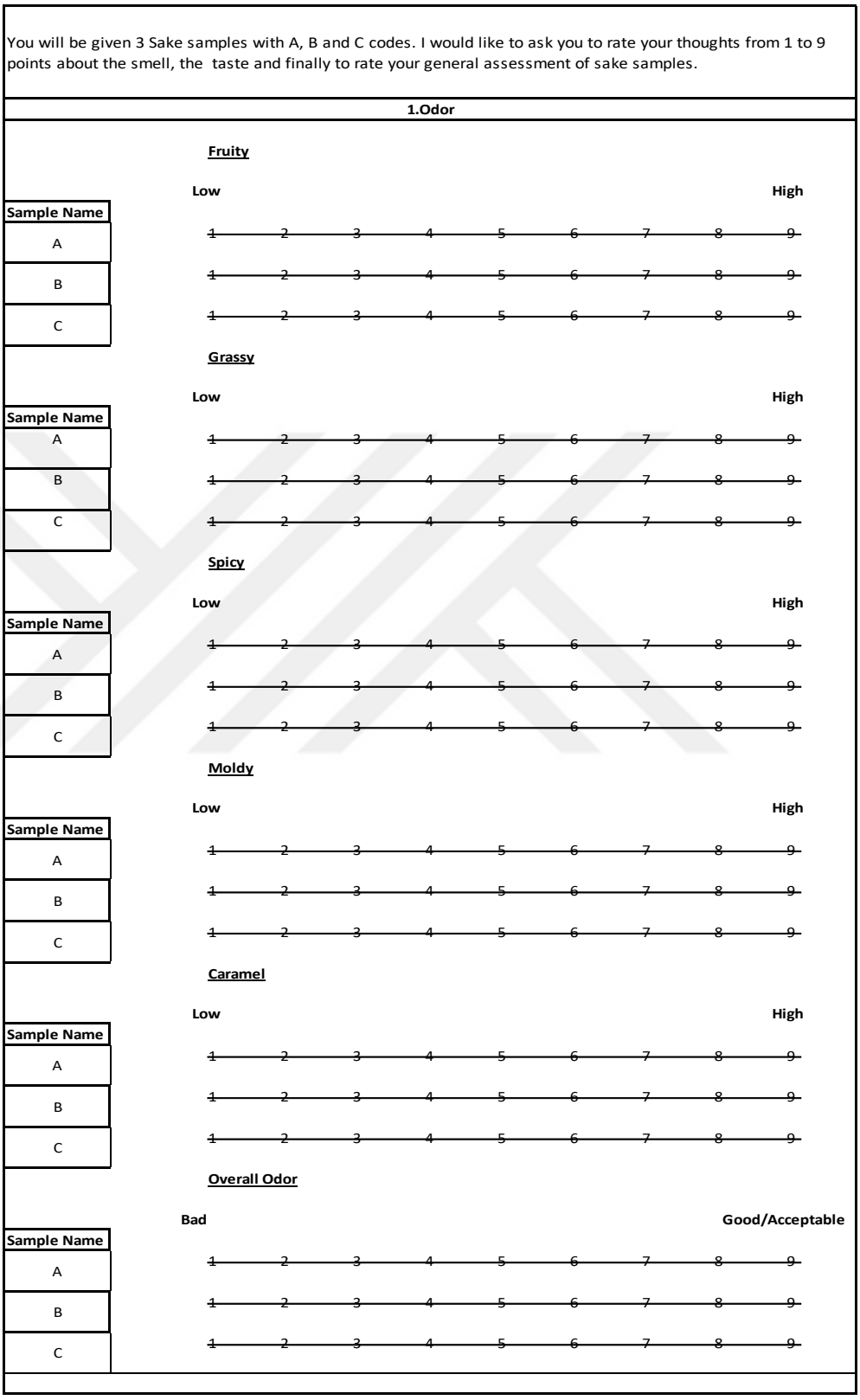
Moldy	Tukey HSD	A	B	1.28000	.55120	.059	-.0391	2.5991
			C	.08000	.55120	.988	-1.2391	1.3991
		B	A	-1.28000	.55120	.059	-2.5991	.0391
			C	-1.20000	.55120	.082	-2.5191	.1191
		C	A	-.08000	.55120	.988	-1.3991	1.2391
			B	1.20000	.55120	.082	-.1191	2.5191
Caramel	Tukey HSD	A	B	.72000	.60074	.458	-.7176	2.1576
			C	.28000	.60074	.887	-1.1576	1.7176
		B	A	-.72000	.60074	.458	-2.1576	.7176
			C	-.44000	.60074	.745	-1.8776	.9976
		C	A	-.28000	.60074	.887	-1.7176	1.1576
			B	.44000	.60074	.745	-.9976	1.8776
O.Odor	Tukey HSD	A	B	.04000	.61319	.998	-1.4274	1.5074
			C	.32000	.61319	.861	-1.1474	1.7874
		B	A	-.04000	.61319	.998	-1.5074	1.4274
			C	.28000	.61319	.892	-1.1874	1.7474
		C	A	-.32000	.61319	.861	-1.7874	1.1474
			B	-.28000	.61319	.892	-1.7474	1.1874
Fullness	Tukey HSD	A	B	.16000	.63337	.965	-1.3557	1.6757
			C	.32000	.63337	.869	-1.1957	1.8357
		B	A	-.16000	.63337	.965	-1.6757	1.3557
			C	.16000	.63337	.965	-1.3557	1.6757
		C	A	-.32000	.63337	.869	-1.8357	1.1957
			B	-.16000	.63337	.965	-1.6757	1.3557

Alcohol	Tukey HSD	A	B	-2.80000*	.59829	.000	-4.2318	-1.3682
			C	-.92000	.59829	.280	-2.3518	.5118
		B	A	2.80000*	.59829	.000	1.3682	4.2318
			C	1.88000*	.59829	.007	.4482	3.3118
		C	A	.92000	.59829	.280	-.5118	2.3518
			B	-1.88000*	.59829	.007	-3.3118	-.4482
Metallic	Tukey HSD	A	B	-.04000	.62638	.998	-1.5390	1.4590
			C	-.64000	.62638	.566	-2.1390	.8590
		B	A	.04000	.62638	.998	-1.4590	1.5390
			C	-.60000	.62638	.606	-2.0990	.8990
		C	A	.64000	.62638	.566	-.8590	2.1390
			B	.60000	.62638	.606	-.8990	2.0990
Sourness	Tukey HSD	A	B	1.12000	.60787	.163	-.3347	2.5747
			C	-.12000	.60787	.979	-1.5747	1.3347
		B	A	-1.12000	.60787	.163	-2.5747	.3347
			C	-1.24000	.60787	.110	-2.6947	.2147
		C	A	.12000	.60787	.979	-1.3347	1.5747
			B	1.24000	.60787	.110	-.2147	2.6947
Sweetness	Tukey HSD	A	B	-1.12000	.62054	.175	-2.6050	.3650
			C	-.28000	.62054	.894	-1.7650	1.2050
		B	A	1.12000	.62054	.175	-.3650	2.6050
			C	.84000	.62054	.371	-.6450	2.3250
		C	A	.28000	.62054	.894	-1.2050	1.7650
			B	-.84000	.62054	.371	-2.3250	.6450

Bitterness	Tukey HSD	A	B	-.24000	.59956	.916	-1.6748	1.1948
			C	-.64000	.59956	.537	-2.0748	.7948
		B	A	.24000	.59956	.916	-1.1948	1.6748
			C	-.40000	.59956	.783	-1.8348	1.0348
		C	A	.64000	.59956	.537	-.7948	2.0748
			B	.40000	.59956	.783	-1.0348	1.8348
MouthFeel	Tukey HSD	A	B	-1.16000	.54340	.090	-2.4604	.1404
			C	-.36000	.54340	.786	-1.6604	.9404
		B	A	1.16000	.54340	.090	-.1404	2.4604
			C	.80000	.54340	.310	-.5004	2.1004
		C	A	.36000	.54340	.786	-.9404	1.6604
			B	-.80000	.54340	.310	-2.1004	.5004
O.Evaluation	Tukey HSD	A	B	-1.28000	.56944	.070	-2.6428	.0828
			C	.12000	.56944	.976	-1.2428	1.4828
		B	A	1.28000	.56944	.070	-.0828	2.6428
			C	1.40000*	.56944	.043	.0372	2.7628
		C	A	-.12000	.56944	.976	-1.4828	1.2428
			B	-1.40000*	.56944	.043	-2.7628	-.0372

*. The mean difference is significant at the 0.05 level.

A22. Sensory Analysis Form



You will be given 3 Sake samples with A, B and C codes. I would like to ask you to rate your thoughts from 1 to 9 points about the smell, the taste and finally to rate your general assessment of sake samples.

1.Odor

Fruity

Sample Name	Low								High
A	1	2	3	4	5	6	7	8	9
B	1	2	3	4	5	6	7	8	9
C	1	2	3	4	5	6	7	8	9

Grassy

Sample Name	Low								High
A	1	2	3	4	5	6	7	8	9
B	1	2	3	4	5	6	7	8	9
C	1	2	3	4	5	6	7	8	9

Spicy

Sample Name	Low								High
A	1	2	3	4	5	6	7	8	9
B	1	2	3	4	5	6	7	8	9
C	1	2	3	4	5	6	7	8	9

Moldy

Sample Name	Low								High
A	1	2	3	4	5	6	7	8	9
B	1	2	3	4	5	6	7	8	9
C	1	2	3	4	5	6	7	8	9

Caramel

Sample Name	Low								High
A	1	2	3	4	5	6	7	8	9
B	1	2	3	4	5	6	7	8	9
C	1	2	3	4	5	6	7	8	9

Overall Odor

Sample Name	Bad								Good/Acceptable
A	1	2	3	4	5	6	7	8	9
B	1	2	3	4	5	6	7	8	9
C	1	2	3	4	5	6	7	8	9

Figure A.22. Sensory Analysis Form

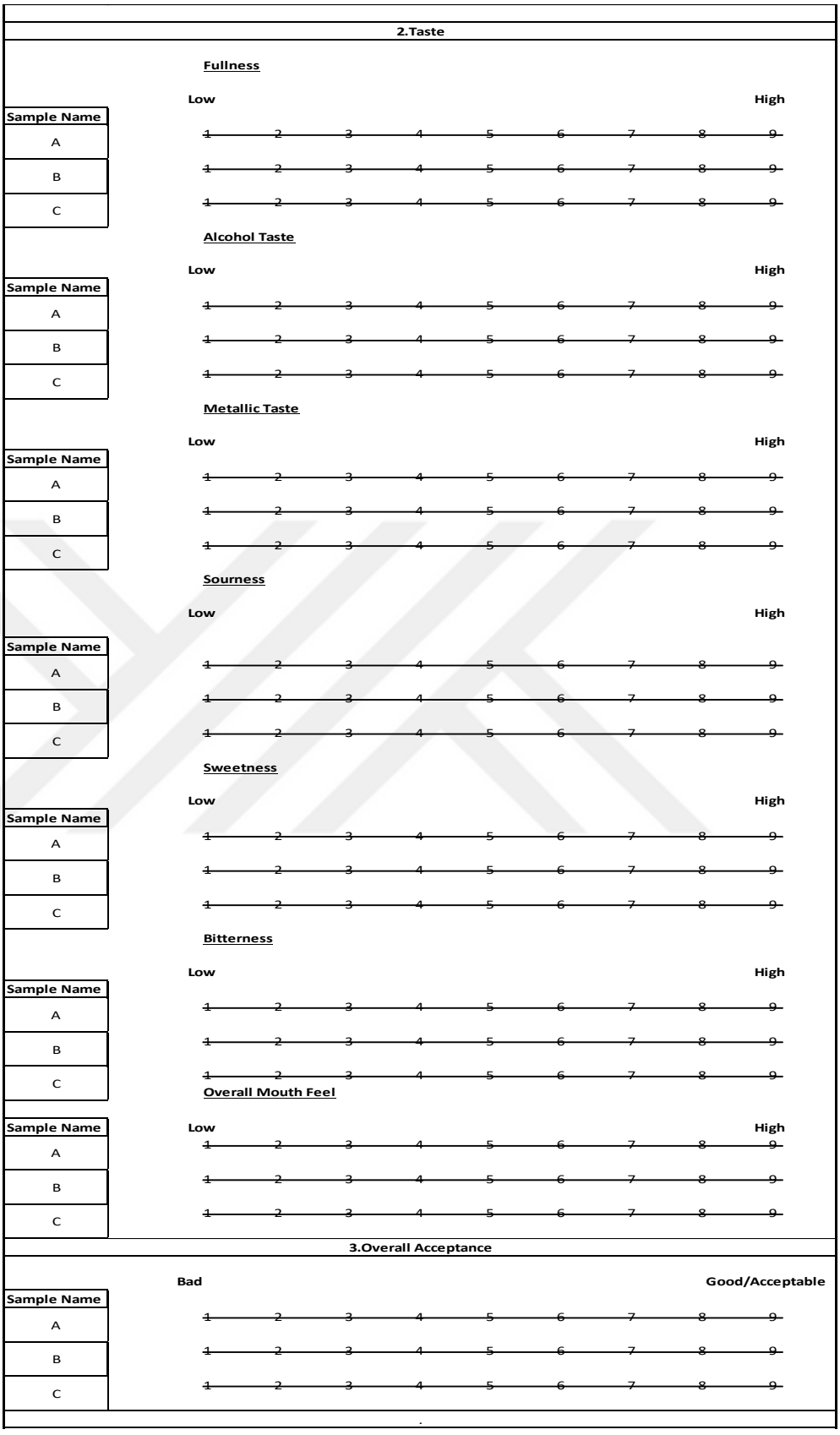


Figure A22 cont'd. Sensory Analysis Form

A23. Sensory Analysis Whole Data

Table A.23. Sensory analyses whole data

		Odor						Taste							Overall Evaluation
	Sample Name	**Fruity**	**Grassy**	**Spicy**	**Moldy**	**Caramel**	**Overall Odor**	**Fullnes**	**Alcohol**	**Metallic**	**Sourness**	**Sweetness**	**Bitterness**	**Overall Taste**	
Panelist 1	A	6	4	5	9	3	9	9	1	1	7	4	1	8	8
	B	5	4	6	4	3	8	6	8	1	4	7	1	7	7
	C	6	4	6	7	4	8	5	5	3	5	5	1	9	7
Panelist 2	A	2	5	5	3	2	2	2	2	2	7	2	3	3	1
	B	6	2	2	2	7	7	7	8	2	1	8	7	8	8
	C	4	4	4	6	2	5	2	2	2	4	2	2	3	4
Panelist 3	A	1	4	2	6	2	5	6	6	6	8	3	7	4	6
	B	7	2	2	4	5	9	8	8	6	4	8	3	8	8
	C	3	4	2	6	2	5	7	9	8	9	2	8	6	7
Panelist 4	A	7	4	4	4	6	5	2	1	2	1	2	2	3	4
	B	5	4	6	6	4	7	5	5	2	5	5	2	5	7
	C	3	4	3	3	6	3	3	2	4	3	3	4	3	4
Panelist 5	A	3	3	4	4	4	3	2	2	1	2	1	3	3	3
	B	2	2	3	3	3	2	6	7	5	3	4	5	6	6
	C	5	5	4	3	3	3	4	5	4	7	5	4	5	5
Panelist 6	A	8	3	4	3	7	8	8	1	8	4	1	6	1	1

	B	1	1	2	2	9	1	4	6	3	1	3	2	6	5
	C	6	2	2	3	7	8	8	1	8	4	1	5	1	1
Panelist 7	A	1	3	1	5	2	2	2	2	6	2	2	2	2	2
	B	2	2	3	4	1	3	3	3	2	2	4	3	3	3
	C	3	4	4	4	3	3	3	5	3	4	5	4	4	4
Panelist 8	A	2	1	1	1	1	3	3	1	1	3	3	1	3	4
	B	3	1	1	1	1	5	4	5	1	1	5	1	5	5
	C	3	1	1	1	1	3	2	1	1	3	2	1	3	2
Panelist 9	A	7	2	3	3	5	7	2	5	4	5	5	5	5	6
	B	6	2	2	2	5	8	2	5	3	3	8	2	6	9
	C	7	2	2	2	5	8	2	5	3	4	8	4	5	5
Panelist 10	A	2	1	7	4	2	6	3	6	7	6	3	8	5	3
	B	8	1	2	1	1	7	3	7	5	4	5	6	7	6
	C	1	6	4	6	2	6	1	5	3	6	4	7	8	8
Panelist 11	A	2	1	1	1	1	4	3	3	3	4	1	6	3	4
	B	1	1	1	1	1	3	3	5	2	2	1	4	6	6
	C	3	1	1	1	1	4	3	2	4	2	1	5	5	3
Panelist 12	A	2	2	5	4	1	2	5	4	3	4	1	1	3	3
	B	5	3	5	1	2	6	4	7	4	2	1	2	6	6
	C	3	5	4	4	1	5	7	5	7	1	1	2	4	6
Panelist 13	A	7	1	1	1	1	9	1	7	8	9	1	8	6	7
	B	7	1	1	1	1	8	1	9	8	6	1	9	7	9
	C	7	1	1	2	1	6	1	9	9	9	1	6	5	6
Panelist 14	A	7	2	3	1	4	6	8	6	7	5	7	4	7	4
	B	5	2	2	1	3	5	4	7	5	4	8	4	6	5
	C	8	2	2	1	5	6	8	6	6	5	7	5	7	6

Panelist 15	A	8	6	7	3	7	7	6	4	3	5	6	4	7	8
	B	5	3	4	2	2	3	6	8	6	7	4	6	7	7
	C	7	7	6	4	4	4	5	3	4	8	5	6	5	5
Panelist 16	A	7	7	2	1	2	8	6	2	1	4	5	1	4	8
	B	3	1	3	3	3	6	4	6	5	2	2	5	5	9
	C	2	3	2	2	5	3	2	2	7	6	4	8	3	3
Panelist 17	A	6	8	7	8	4	5	7	8	3	4	3	4	7	7
	B	4	3	2	1	3	4	6	9	7	6	6	5	6	6
	C	3	6	6	7	3	6	5	6	5	5	5	5	5	5
Panelist 18	A	6	5	1	7	2	7	8	7	4	8	2	7	5	6
	B	5	1	1	1	1	6	6	8	2	3	1	7	7	8
	C	1	2	1	5	2	6	5	5	4	6	2	5	5	4
Panelist 19	A	2	6	5	6	5	6	4	2	2	1	6	3	6	5
	B	3	4	2	4	1	7	5	5	6	3	5	5	7	6
	C	4	7	6	5	6	5	6	4	4	4	6	6	7	7
Panelist 20	A	6	3	6	4	7	7	8	6	2	6	2	6	8	8
	B	4	3	3	2	5	5	7	8	2	6	2	8	6	6
	C	5	3	6	4	7	7	7	6	2	6	2	6	7	7
Panelist 21	A	7	5	8	2	2	2	3	4	2	6	6	3	2	2
	B	2	4	4	6	2	3	4	6	2	5	7	3	4	4
	C	3	3	5	4	2	1	6	9	2	3	5	3	8	7
Panelist 22	A	8	3	2	2	7	2	5	7	2	1	3	3	8	8
	B	6	5	4	1	2	5	3	5	1	1	4	2	6	6
	C	4	7	7	1	3	3	4	6	1	1	2	3	7	5

Panelist 23	A	7	7	8	4	8	8	6	2	2	2	7	2	8	8
	B	4	5	3	2	2	3	4	8	3	2	2	4	3	3
	C	5	6	4	3	4	6	5	5	2	3	6	3	6	5
Panelist 24	A	3	2	5	1	1	5	1	1	1	3	2	3	5	4
	B	4	1	3	1	2	7	1	5	1	2	3	4	4	6
	C	2	1	6	2	1	6	2	7	2	1	1	7	2	2
Panelist 25	A	2	3	2	2	2	3	2	4	4	2	1	4	1	3
	B	1	1	3	1	1	2	2	6	2	2	3	3	5	4
	C	1	1	3	1	1	3	1	2	3	3	1	3	3	2