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Effects of Temperature, Mother of Vinegar, and Amount of Sugar on Aronia melanocarpa Chokeberry Fermentation

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Keywords: *Vinegar, Aronia, melanocarpa Chokeberry Fermentation etc.*

Abstract

This study evaluatesthe effects of temperature, mother of vinegar, and amount of sugaron the fermentation of black chokeberry (Aroniamelanocarpa). To investigate temperature, samples of black chokeberrieswere compared after fermentation in a thermostat incubator (T 40^{o}C) and at room temperature (T 25^{o}C). Six mothers of vinegar were also used: white grape vinegar, brown rice black vinegar (pomegranate), brown rice black vinegar (blackberry and blueberry), pomegranate vinegar, brown rice vinegar, and white rice vinegar. The amounts of sugar were 150g and 350g. The fermentation is divided into periods of alcoholic fermentation and vinegar fermentation. Evaluations were done by analyzing the pH value, Brix development, and microbial visual observation. The development of pH and sugar content in the vinegar influence the general bacteria and acetic acid bacteria in the early fermentation stage, while they influence the yeast in the second period of fermentation. The samples were also analyzed under microscope, whichshowed that the black chokeberries predominantly contained Gram-negative bacteria. The quality of the vinegar is greatly affected by the storage temperature. The suitable temperature for fermentation wasdetermined to be 40°C.

INTRODUCTION

Considering their benefits and their availability in the Republic of Korea, chokeberry (*Aroniamelanocarpa*) has potential for use in fruit vinegars. Chokeberry polyphenols have been marketed for their potential in decreasing the risk of heart disease through their antioxidant and anti-inflammatory activity. Chokeberry consumption also has a protective effect on some body tissues, includingthe lung, kidney, and liver. These effects are believed to be related to its high concentrations of phenolic compounds, including anthocyanins and proanthocyanidins [1]. Flavonols are also known to have direct inhibitory activity on some viruses.

Vinegar is a condiment made from various sugary and starchy materials by alcoholic and subsequent acetic fermentation [2]. Vinegar plays is used in salad dressings, ketchup, hot sauce, and other sauces. Industrial fermentation systems are needed produce largesamount of vinegar. These systems must maintain reliable controls and optimum conditions for acetic acid bacteria fermentation [3]. Many techniques have been developed to improve the industrial production of vinegar. Most try to increase the speed of the transformation of ethanol into acetic acid in the presence of acetic acid bacteria [4]. The most common technology for the vinegar industry is based on submerged cultures [5] with various technical modifications meant to improve the general fermentation conditions (aeration, stirring, heating, etc.).

In the last few years, the use of vinegar as a food ingredient and functional beveragehas increased [6]. Research on chokeberry vinegar usually focuses on two periods: the first period of chokeberry and mother of vinegar fermentation to produce chokeberry wine (alcoholic fermentation), and the second period of fermenting this wine with added sugar to produce vinegar (vinegar fermentation). Several factors affect yeast growth during alcoholic fermentation, including clarification of the chokeberry juice, the addition of sulfur dioxide, fermentation temperature, chokeberry juice composition, inoculation with selected yeasts, and interactions with other organisms[7].

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Wines are often fermented in the range of 10–20°C. However, some European wineries prefer fermentation temperatures between 20 and 25°C. In recent years, there has been a preference by some winemakers to ferment white wines at lower temperatures to enhance the production and retention of flavor volatiles. Such trends have required the selection and use of *Saccharomycescerevisiae*strains, which exhibit good growth rates at low temperatures [8] [7]. The temperature of fermentation can affect the development of different *Saccharomyces* strains. The yield of ethanol and other fermentation byproducts is also related to temperature [9].

Temperature can affect the sensitivity of yeasts to alcohol concentration, growth rate, rate of fermentation, viability, length of lag phase, and enzyme and membrane function. Because yeast strains differ in response to temperature, the optimum temperature for vinification can vary widely [7]. Fermentation temperature is known to influence wine aroma composition [10]. Low temperature fermentation in particular has been reported to improve the taste and aroma of wine and to increase ethanol and beer productivity [11] [12].Since fermentation temperature affects the quality of the wine produced from a given cultivar, there may be an optimum temperature for the best results.The objective of this study is to analyze the effect of temperature on chokeberry fermentation to optimize the fermentation time and promote the chokeberry as an economical source of fermentable berries.

MATERIALS & METHODS

Preparation of chokeberry sampling

The initial pH and sugar content of chokeberries are 3.9 pH and 13.7 degrees Brix. Six different mothers of vinegar were used, 350ml of which were added to the fermentation process.Table 1 shows the various sugar contents and the fermentation temperatures.

Table 1Sample ingredients for black chokeberry vinegar fermentation

Mother of vinegar (350 ml)	Tag	Sugar (g)	T (°C)	Mother of vinegar (350 ml)	Tag	Sugar (g)	T (°C)
Treatment **A** White grape vinegar	A1	150	40	Treatment **D** Pomegranate vinegar	D1	150	40
	A2	150	25		D2	150	25
	A3	350	40		D3	350	40
	A4	350	25		D4	350	25
Treatment **B** Brown rice black vinegar ; pomegranate	B1	150	40	Treatment **E** Brown rice vinegar	E1	150	40
	B2	150	25		E2	150	25
	B3	350	40		E3	350	40
	B4	350	25		E4	350	25
Treatment **C** Brown rice black vinegar ; blackberry & blueberry	C1	150	40	Treatment **F** White rice vinegar (handmade)	F1	150	40
	C2	150	25		F2	150	25
	C3	350	40		F3	350	40
	C4	350	25		F4	350	25

pHand brix development analysis

The pHwas measured by a pH-meter(ISTEK, Inc. Type: pH-250L, Korea) during 15 days of the fermentation process. Brix analysis of the sugar content was conducted using a portable refractometer (ATAGO Type: PAL-1, Japan). Drops of sample were placed onto a refractometer slide and held at 30º towards a light source to measure the Brix sugar content. The solution of chokeberries was analyzed in triplicate to determine an average Brix value for each juice sample. The pH level and degrees Brix were monitored every 12 h during the fermentation period.

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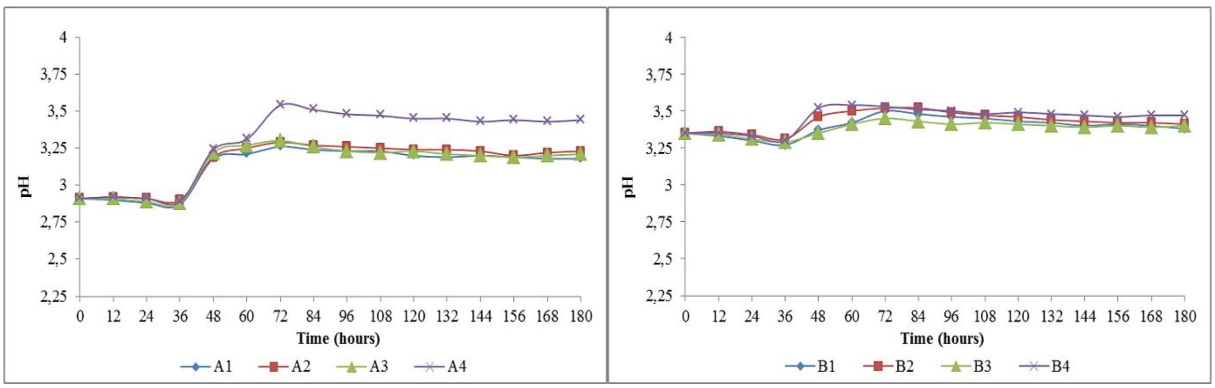
Microbial visual observation analysis

An inverted optical microscope was used to observe the microbial growth (TS100LED ECLIPSE MV, Nikon, Japan).The microscope was equipped with a micro camera and connected to a computer to simplify the observation process.The devices were used to observe the microbial growth in the initial conditions and in the middle and final stagesof the fermentation process

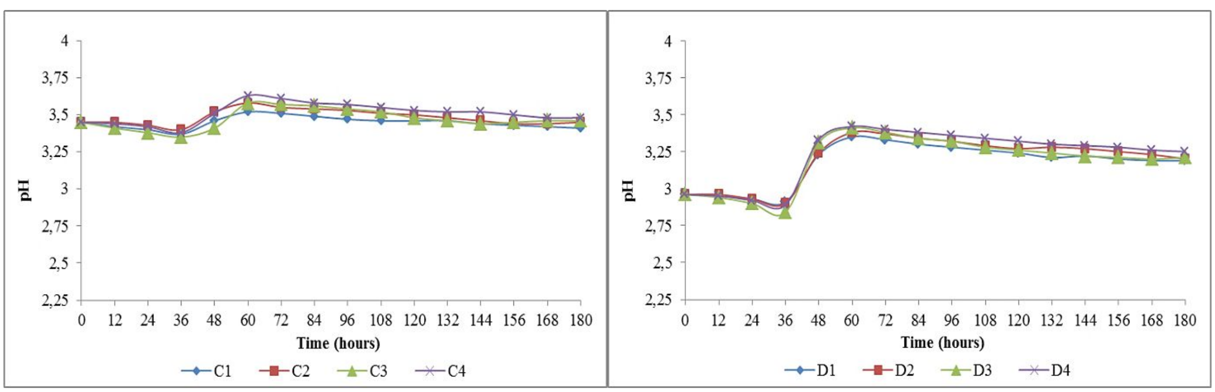
RESULTS & DISCUSSIONS

pH development during fermentation

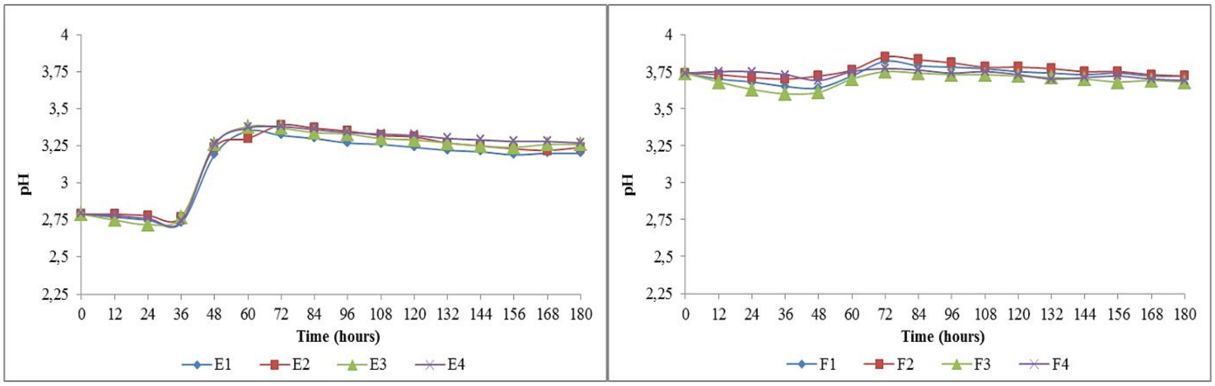
The pH of vinegar depends on how much acid is present. Fermentation proceeds appropriately when the pH of the mash is adjusted to the range of 3.0 to 4.5, which is favorable for yeast growth. As the concentration of the total solids decreases, the pH increases. The changes in pH aredue to changes in the concentrations of sugars and organic acids. As shown in Figure 1,the initial pH with different mothers of vinegar is high after the addition of granulated sugar, so it was necessary to adjust it to the optimum pH for the growth of the yeast during fermentation.



(a) White grape vinegar (b) Brown rice black vinegar; pomegranate



(c) Brown rice black vinegar; blackberry & blueberry (d)Pomegranate vinegar



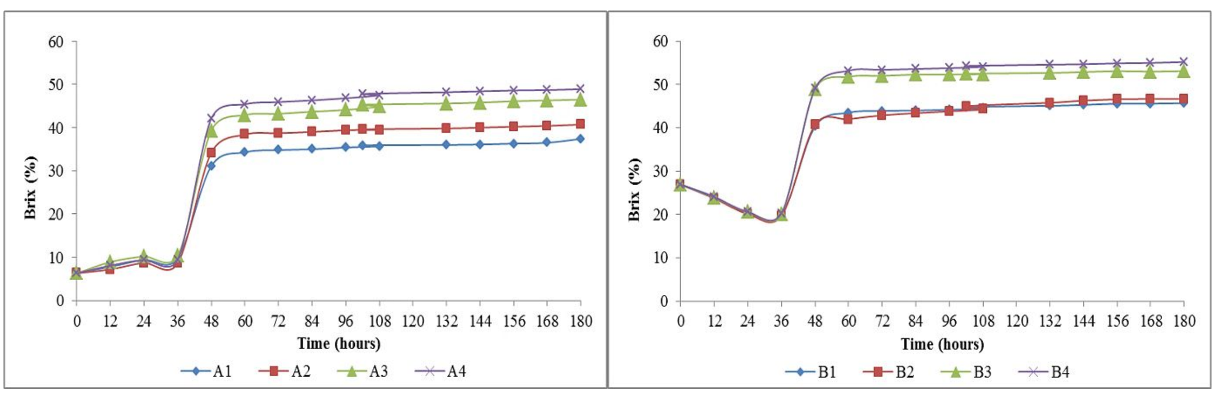
(e) Brown rice vinegar ***(f) White rice vinegar***

Figure 1.pH development during fermentation with the different mothers of vinegar

Degrees Brix during chokeberry fermentation

Vinegar is a dilute solution of acetic acid that results from a two-step fermentation process. The first step is the fermentation of sugar into alcohol, usually by yeast. Any natural source of sugar can be used. For example, the sugar may be derived from the juiceof a fruit (such as grapes, apples, raisins, or even coconuts),or from a grain (such as barley or rice), honey, molasses, sugar cane, or even cellulose in wood (such as beech) in the case of certain distilled vinegars. Traditional vinegar is produced from raw materials containing sugar or starch in a two-stage fermentation to produce ethanol and then acetic acid. Vinegar production typically involves a first fermentation where simple sugars in the raw material are converted to alcohol by yeasts.

As the alcoholic fermentation proceeds, the amount of sugars decreases as alcohol is produced (0–36h). The content of individual sugars in chokeberry juice was not measured in this study, but the relative sugar contents of samples were determined every 12 h through Brix analysis.A gradual increasing trend was observed, as shown in Figure 2. The percentage of reduced sugars is high because all the sucrose is converted to the reduced sugarsglucoseand fructose. The high concentration of sugar inhibits the growth of microorganisms.



(a) White grape vinegar ***(b) Brown rice black vinegar; pomegranate***

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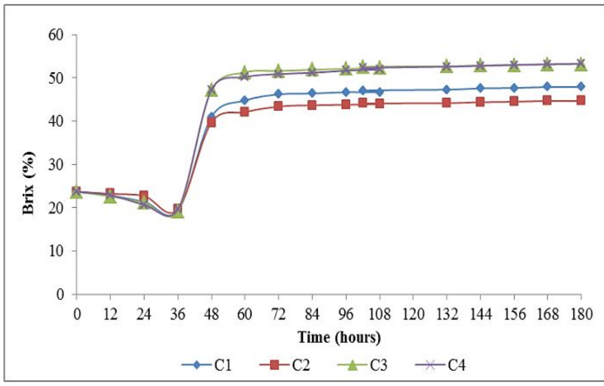
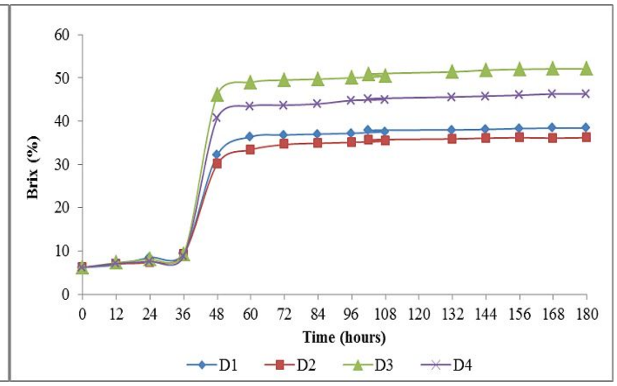
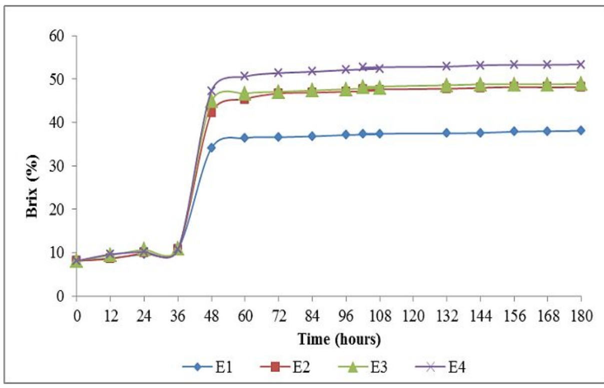


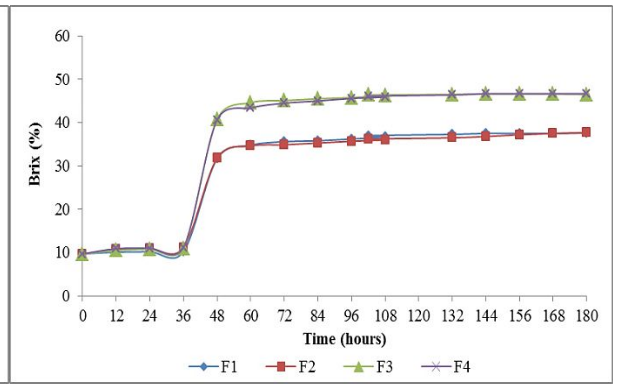
(c) Brown rice black vinegar; blackberry & blueberry



(d) Pomegranate vinegar



(e) Brown rice vinegar

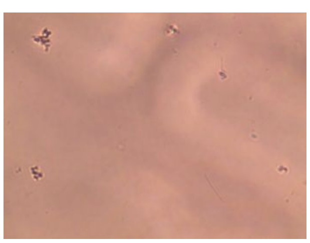


(f) White rice vinegar

Figure 2.DegreesBrix behavior during fermentation with different mothers of vinegar

Microbial growth observation

The bacterial strains, temperature, pH, acid concentration, and ionic strength influence the antimicrobial activity of organic acids in vinegar. After sampling, the slide was mounted under a microscope with 10x100 magnification. The pink color indicates Gram-negative character of the bacteria, while a blue color indicates Gram-positive. The vinegar cultures are predominantly Gram-negative bacteria, as shown in Figure 3.



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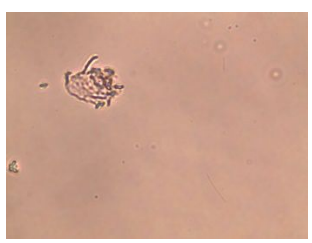

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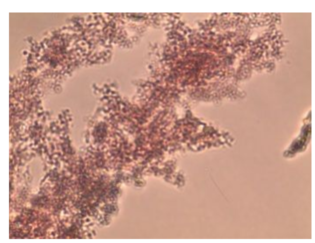

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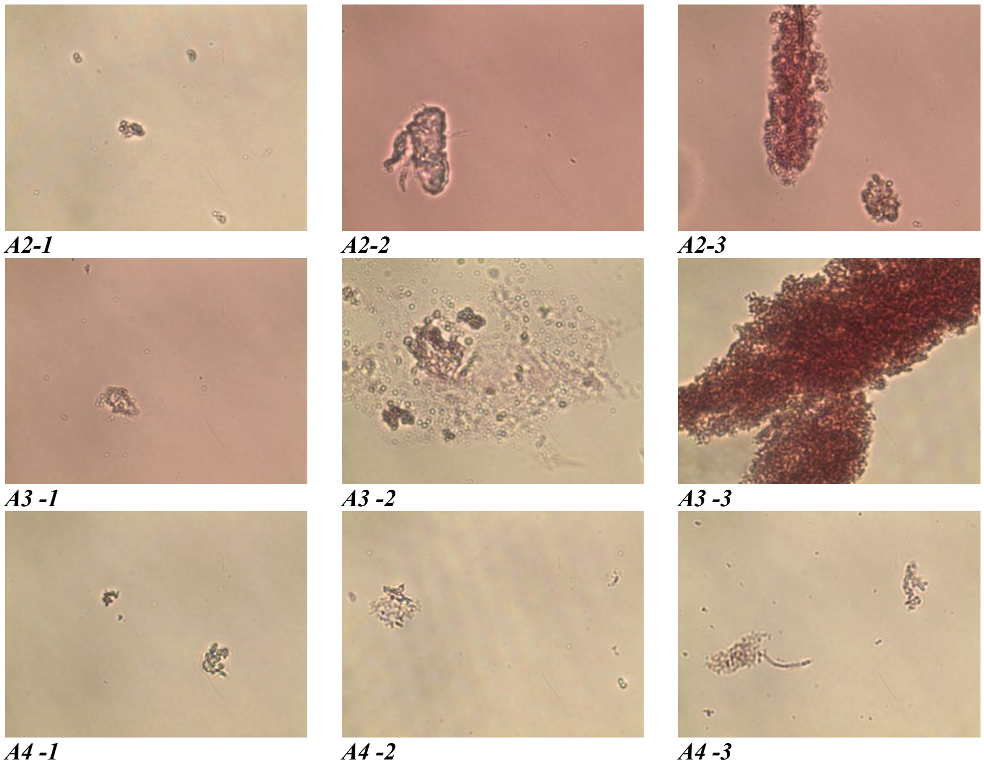

Figure 3. Microbial observation of the fermentation of black chokeberries with white grape mother of vinegar; 1= initial condition, 2=growth condition, 3=optimum growth condition

CONCLUSION

Ripened black chokeberries may be an economical commercial source of fermentable berries for manufacturing vinegar in countries that produce black chokeberries. The development of pH and sugar content in the vinegar influence the general bacteria and acetic acid bacteria in the early fermentation stage, while they influence the yeast in the second period of fermentation. Microscope analysis of the samplesshowed that the black chokeberries predominantly contain Gram-negative bacteria. The quality of the vinegar is greatly affected by the storage temperature,which is essential to control in order to address the problems that occur from storage. The recommended temperature for fermentation was shown to be 40°C.

REFERENCE

1. *C. Zhao, M. Guisti, M. Malik, M. Moyer and B. Magnuson, " Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth," J. Agric. Food Chem., vol. 52, pp. 6122-6128, 2004.*
2. *W. Cruess, Commercial fruit and vegetable products, 1st ed ed., vol. Chapter 21 – Vinegar manufacture, New York: McGraw-Hill Book Company, Inc., 1958, pp. p 681-707..*
3. *I. De Ory, L. Romero and D. Cantero, "Maximum yield acetic acid fermenter. Comparative fed-batch and continuous operation studies at pilot plant scales," Bioprocess Engineering, vol. 21, pp. 187-190, 1999.*
4. *W. Tesfaye, L. Morales, M. Gacia-Parrilla and A. Troncoso, "Wine vinegar:technology, authenticity and quality evaluation," Journal of Food Science and Technology, vol. 13, pp. 12-21, 2002.*
5. *O. Hormatka and H. Ebner, "Enzymology," J Biotechnol, vol. 15, pp. 57-69, 1951.*

6. *O. Adachi, E. Miyagawa, E. Shinagawa, K. Matsushita and M. Ameyama, Purification and properties of particulate alcohol dehydrogenase from Acetobacter aceti. Agricultural and Biological Chemistry, vol. 42, 1978, pp. 2331-2340.*
7. *R. Jackson, Wine Science, Academic Press, 2000.*
8. *G. H. Fleet and G. M. Heard, Yeast: Growth During Fermentation. In: Wine Microbiology and Biotechnology, Harwood Academic Publisher, 1993.*
9. *M. J. Torija, N. Rozes, M. Poblet and J. G. a. A. Mas, "Effect of Fermentation Temperature on The Strain Population of Saccharomyces cerevisiae," International Journal of Food Microbiology, vol. 80, pp. 47-53, 2003.*
10. *A. Bekatorou, A. Sarellas, N. Ternan, A. Mallouchos, M. Komaitis and A. a. K. M. Koutinas, "Low Temperature Brewing Using Yeast Immobilized on Dried Figs," Journal of Agricultural and Food Chemistry, vol. 50, no. 25, pp. 7249-7257, 2002.*
11. *E. P. Bardi, A. A. Koutinas, M. J. Soupioni and M. E. Kanellaki, "Immobilization of Yeast on Delignified Cellulosic Material for Low Temperature Brewing," Journal of Agricultural and Food Chemistry, vol. 44, no. 2, pp. 463-467, 1996.*
12. *E. P. Bardi and A. A. a. K. M. Koutinas, "Room and Low Temperature Brewing With Yeast Immobilized on Gluten Pellets," Process of Biochemistry, vol. 32, no. 8, pp. 691-696, 1997.*