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Research Article

Study on Influencing Factors of the Activity Assay for Glucoamylase of Koji

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Abstract: To confirm the most correct method between different analysis methods used in the activity analysis of glucoamylase of Koji, the analysis result by several methods used commonly were compared in this study. It is showed that results were quite different between different measurement methods. Glucoamylase activity in Koji by white spirit method was 36~200 u/g; it was 144.98~318.95 u/g by the method of national standard of China (GB), it was 26.96~146.67 u/g by the DSN method, it was 392.04~419 u/g by Japanese Sake Koji analysis method. It was particularly evident in the sample amount for GB; results by enzymes extraction at 30°C were greater than that at 40°C. By different enzyme extraction methods, the result show that, the activity with the extraction method in sake was greater than that with other two extraction methods of GB and DSN. By different measurement methods, but using same extraction method, the result show measurement methods in several assays had some adaptability. In shortly, glucoamylase activity by the method used in Sake was the most stable, best embodying. Activity of glucoamylase by the extraction method (by dialysis), measuring with DNS measurement method, the results were relatively stable, closest to it.

Keywords: Assay, extraction, GB, glucoamylase, koji, liquor koji, rice-koji

INTRODUCTION

Koji is one of the important raw materials in production of Chinese traditional liquor, Japanese sake, soy sauce, through providing various enzymes and the necessary nutrient source for yeast during fermentation and plays an important contribution in giving the liquor a variety of flavors substances (Xu and Xu, 1998). Rice-koji is made from rice and it can produce glucoamylase, amylase proteolysis enzyme, etc. during Koji production. It can decompose soybeans, rice, wheat, sorghum, maize, etc. into glucose and amino acids. The brewing process mainly depends on the ability of enzymes and transferring the raw material starch into glucose, therefore, the decomposition of the raw material is largely determined by level of enzyme activity of glucoamylase (Xie and Yang, 2009).

In Chinese traditional liquor production, the enzymes and nutrients in koji promote the proliferation of yeast and fermentation, in which saccharification glucoamylase is real factor (Glucoamylase, EC3.2.1.3), referred glucoamylase (Guan *et al*., 1993; Zhu *et al*., 2012), one kind of exo-glycosidases, it can sequentially hydrolyze α-1,4 glycosidic bond in the non-reducing end of the steamed rice starch, cut a glucose unit and like the β-amylase, conformationally change the hydrolysis-downed glucose, formatting β-D-glucose. When faced with a branch point of amylopectin, it can also hydrolyze α-1, 6 glycosidic bond, whereby totally hydrolyzing the pullulan into glucose. Glucoamylase also weakly hydrolyze α-1,3 glycosidic bonds, which generally can 100% hydrolyze starch into glucose (Wujin *et al*., 2002) and then glucose is fermented by yeast into alcohol, koji glucoamylase activity is crucial for wine production.

Glucoamylase extraction methods include liquor koji glucoamylase extraction, GB glucoamylase enzyme extraction method, the measurement methods of glucoamylase activity include, GB Iodometry, white spirit Fehling reagent, DNS in soy sauce production. In this study, we conduct a comparative study on the several glucoamylase extraction and activity measurement methods, but also compared with the extraction method and activity measurement method of the Japanese sake koji, in order to find a rapid and accurate measurement method of rice-koji glucoamylase activity.

MATERIALS AND METHODS

Aspergillus oryzae A52820131203 preserved in brewing wine laboratory. Potato Dextrose Agar (PDA)

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medium: potato 200 g, glucose 20 g, agar 15~20 g, water 1000 mL, pH naturally. Indica, which is commercially available; brewing water, which is in line with GB5749-2006 (drinking water health standards), Soluble starch, sodium iodide, potassium iodide, concentrated sulfuric acid, glacial acetic acid, sodium acetate, sodium chloride, sodium thiosulfate, sodium carbonate, glucose, sulfate, methylene blue, sodium tartrate, ferric K, 3, 5-dinitrosalicylic acid, phenol, sodium sulfite.

Rice-koji production process:

Raw materials (indica rice) wash→soak →dry→pack into flask→ Steamed rice
↓
Measure enzyme activity←stop culturing←buckle bottle←culturing←inocultion←cool

Process operating instructions:
Wash rice: Remove impurities attached to the broken surface, such as chaff, dust and inclusions.

Dip rice: Due to the poor indica rice water absorption, it takes a long time to soak, soak for 12 h or more under the conditions of 20°C. When in appearance grain clarity disappeared into pure white, grind grain with hand and can milled rice, remove the indica rice, drain for 0.5 h then repackage steamed rice.

Steamed rice: With high-pressure steam sterilization pot, 110°C, 45 min. So steamed long rice were cooked but not rotten, without Bai Xin meters.

Inoculation: Aseptic manner in accordance with the appropriate inoculation of Aspergillus oryzae rice-koji. Buckle bottle: Aspergillus oryzae is an aerobic microorganisms (Zhao and Xu, 2006), the mycelium will grow intertwined, causing grain agglomeration, resulting in hypoxia inside rice-koji material, so we should buckle bottles, beaten the agglomerated rice. Rice-koji culturing: the first day after vaccination is the Aspergillus oryzae spore germination stage, the temperature is 31°C, relative humidity of 75%, the second day since the incubation, temperature is 37°C, relative humidity of 75%, generally at the end of the culturing, it can be observed visually a large grain of rice wrapped in a white mycelium.

Glucoamylase liquid extraction: Liquor koji glucoamylase extraction (hereinafter abbreviated white spirit extraction) (Shen, 2014): Weigh equivalent of 5 g dry yeast powder rice-koji into 250 mL beaker, add water (90~5×moisture %) mL, buffer solution 10 mL, leaching bath at 30°C 1 h, stir once every 15 min and then filtered with a dry filter paper, discard the first 5 mL, receive 50 mL clear filtrate standby.

National standard of enzyme preparations used in glucoamylase activity assay extraction (hereinafter abbreviated GB extraction method) (GB8276, 2006): Use 50 mL small beaker accurately weigh appropriate amount of enzyme sample, accurated to 1 mg, dissolved with a small amount of acetic acid-sodium acetate buffer and carefully mashed with a glass rod, the supernatant was carefully poured into a suitable volumetric flask, then add a small amount of the sediment in sodium acetate buffer, repeatedly pounded 3~4 times and remain the supernatant and finally all moved into flask with acetic acid sodium acetate buffer volume, magnetic stirring for 30 min to mix well, then the supernatant was measured. Sake Aspergillus glucoamylase extraction (hereinafter abbreviated sake extraction) (Brewing Society of Japan, 1993): Weigh 2 g absolutely dry koji, add 10 mL of 0.5% NaCl containing 10 mM acetate buffer (pH5.0), the extraction overnight at 4°C in refrigerator, filtered with filter paper (the extraction rate of 5 times) and the filtrate was diluted with distilled water twice, namely test enzyme solution. Weighing 5 g of oven dry koji, added 25 mL of 0.5% NaCl containing 10 mM acetate buffer (pH5.0), extraction at 4°C refrigerator overnight, filtered with filter paper (the extraction rate of 5 times) and the filtrate was diluted with distilled water twice, namely test enzyme solution.

Glucoamylase activity assay: Definition of glucoamylase activity in national standard of enzyme preparations used in glucoamylase activity assay extraction (referred GB assay) (GB8276, 2006): 1 mL enzyme solution or 1 mg enzyme powder at 40°C, pH 4.6, 1 h hydrolysis soluble starch, generates 1 mg glucose, is a unit of enzyme activity in U/mL or u/g. Definition of glucoamylase activity in soy sauce brewing (Leng, 2004) using 3, 5-dinitrosalicylic acid method (referred to DNS assay, Leng (2004)): at 40°C, pH4.6, catalyzed by 1 mg of glucose per minute is defined as an amount of enzyme activity unit, u/g. Definition of glucoamylase activity in glucoamylase activity assay in liquor koji used Fehling reagent (Shen, 2014) in the: 1 g dry koji at 40°C, pH 4.6, 1 h soluble starch decompose generates 1 mg glucose, is a unit of enzyme activity in u/g. Sake Aspergillus glucoamylase determination kit method (hereinafter abbreviated kit method) (Brewing Society of Japan, 1993; Zhou, 1998): Substrate solution 0.5 mL and enzyme solution 0.5 mL were added to a small test tube, preheated at 37°C for 5 min; Diluted rice-koji extract was added 0.1 mL, mixed well to start the reaction, 37°C for 10 min; 2.0 mL of reaction stop solution was added, fully mixed so that the reaction is stopped; The reaction stopping solution absorbance was measured at 400 nm (Es values). (Control is distilled water); Blank measurement, the above-mentioned substrate solution and the enzyme

solution was reacted at 37°C for 15 min, the reaction was stopped by adding solution 2 mL, 0.1 mL added to the sample solution after mixing, the absorbance was measured (Eb value) after mixing; Saccharification force calculation: Calculation method of rice-koji saccharification force saccharification = (Es-Eb)× 0.172×Df×extraction rate = (Es-Eb)×0.171×2×5 = (Es-Eb)×1.71 (Df: dilution ratio = 2; extraction ratio = 5); Referring to Japan's National Tax Agency analysis, glucoamylase activity (u/g song) = 144.6× saccharification.

RESULTS AND DISCUSSION

Influencing factors of white spirit-koji-extraction method: Weigh 2 g, 5 g koji (absolute dry weight) respectively, by dilution white spirit extraction glucoamylase (20-fold dilution) add water, buffer, 30°C water bath leaching 1 h, stir once every 15 min, then dry filter paper, discard the first 5 mL, the filtrate is the test enzyme solution. The results of measuring white spirit song glucoamylase determined by Fehling reagent are shown in Fig. 1.

From the Fig. 1, using extraction method of white spirit koji, Fehling reagent assay, 5 g dry value measured amount of sample curvature slightly greater than the value of the measured amount of sample 2 g, the reason may be because the amount of sample increases, the error is reduced.

Effect of dilute times: Since the above-described method, the measured values of 20-fold diluted extract is small, If increase dilution, the measured value will be smaller, greater errors. Therefore, to reduce the experimental error, reduce the dilution of 10 times. Weigh 2 g, 5 g koji (absolute dry weight) respectively, the extract dilution is 10 times, in accordance with the corresponding proportion add water, buffer, leaching 1 h in 30°C water bath, stir once every 15 min, then with a dry filter paper, discard the first 5 mL, the filtrate is the test enzyme solution. The results of measuring white spirit song glucoamylase determined by Fehling reagent are shown in Fig. 2.

From the Fig. 2, the larger the impact of dilution on the experimental results, 2 g sample volume measured by the amount of sample values greater than 5 g measured values. The reason may be due to reduced dilution, some extract concentration is too high, some substances can not leach out, affect the experimental results.

Effect of extraction temperature:

Extraction temperature: Because GB glucoamylase enzyme activity in the measured temperature is 40°C, in order to study the impact of different extraction temperature on this extraction method, the extraction temperature is 40°C. Weigh 2 g, 5 g koji (absolute dry weight) respectively, extracts by dilution (20-fold

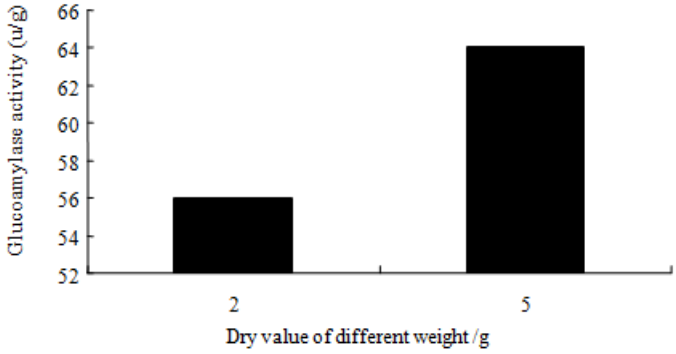

Fig. 1: The measurement results of Fehling reagent (extraction diluted 20-fold)

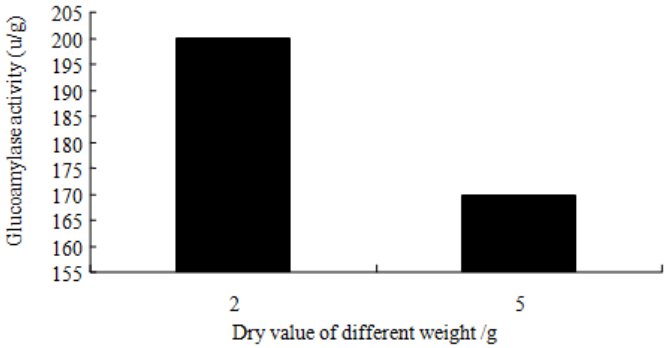

Fig. 2: The measurement results of Fehling reagent (extraction diluted 10-fold)

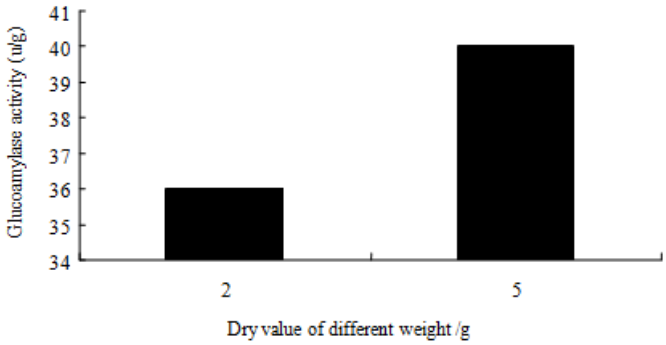

Fig. 3: The measurement results of Fehling reagent (extraction temperature of 40°C)

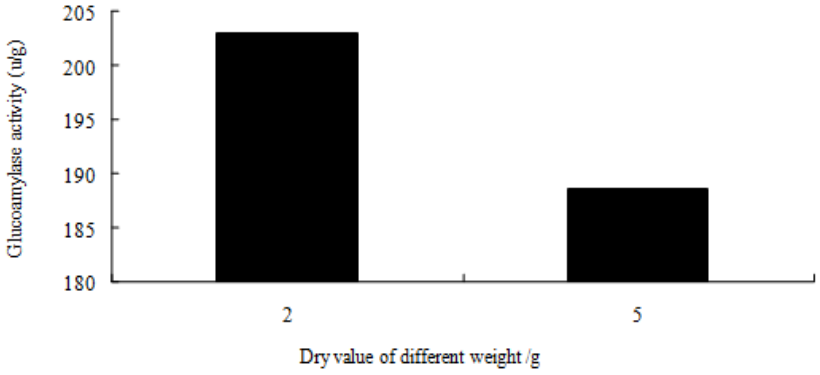

Fig. 4: The measurement results of GB method (extraction diluted 20-fold)

dilution) add water, buffer, extraction temperature is 40°C, then water bath leaching 1 h, stir once every 15 min, then filtered with a dry filter paper, discard the first 5 mL, the filtrate is the test enzyme solution. The results of measuring white spirit koji glucoamylase determined by Fehling reagent are shown in Fig. 3.

From the Fig. 3, 5 g of the sample volume is slightly larger than the measured value of the measured amount of sample values 2 g, show the amount of the sample at this temperature has little effect on the results. To sum up, the determination of glucoamylase activity rice-koji white spirit soaked Tifafeilin reagent use, extract dilution greater than the extraction temperature impact on the experimental results, 20-fold diluted extract is a more appropriate concentration.

Influencing factors of national standard method (GB):

Effect of dilute times: Weigh 2 g, 5 g koji (absolute dry weight) respectively, add acetic acid sodium acetate buffer, dilute 20-fold extract, leaching in water bath at 30°C 1 h, stir once every 15 min, then with a dry filter paper, discard the first 5 mL, the filtrate is the test enzyme solution. Glucoamylase activity was measured according to the national standard of enzymes used national standard method (referred to as the GB method) results shown in Fig. 4.

From the Fig. 4, 2 g sample volume measured by the amount of sample values greater than 5 g measured value, the reason may be because the extraction method extracts all buffer Baptist propose certain other substances, affecting the experimental results.

Effect of extraction temperature: Weigh 2 g, 5 g koji (absolute dry weight) respectively, the extract dilution to 10 times, add sodium acetate buffer solution, extraction in water bath at 30°C 1 h, stir once every 15 min, then filter with a dry filter paper, discard the first 5 mL, the filtrate is the test enzyme solution. The measurement results according to the national standard method GB glucoamylase enzyme activity assay preparations used in Fig. 5.

From the Fig. 5, the measured value increased to reduce the dilution ratio and 2 g sample volume measured by the amount of sample values greater than 5 g measured value, the reason may be because the extract concentration increases, baptism raised more substance, leading to increased results.

Weigh 2 g, 5 g koji (absolute dry weight) respectively, add sodium acetate buffer solution, extraction 20-fold diluted solution, extraction temperature 40°C, then water bath leaching 1 h, stir once every 15 min, then filter with a dry filter paper, discard the first 5 mL, the filtrate is the test enzyme solution. The measurement results according to the

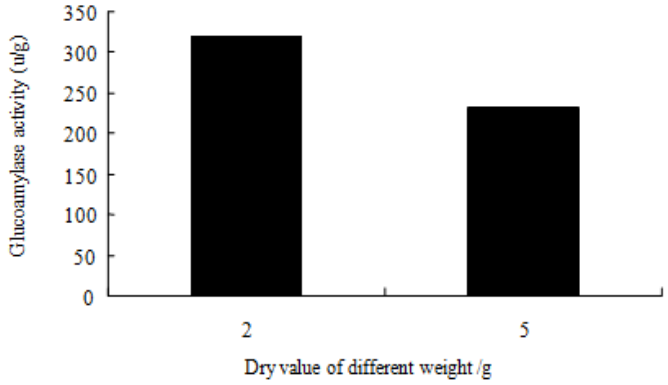

Fig. 5: The measurement results of GB method (extraction diluted 10-fold)

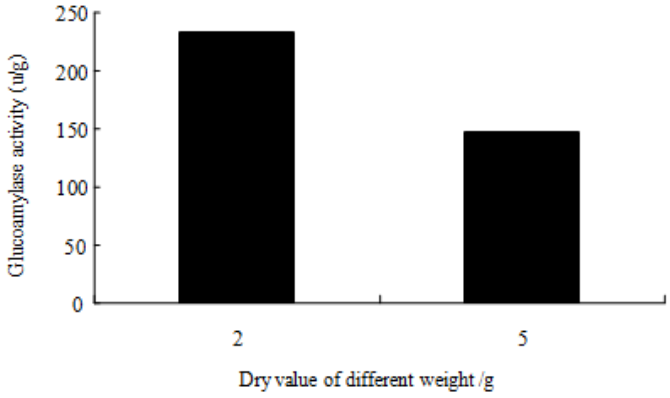

Fig. 6: The measurement results of GB method (extraction temperature of 40℃)

national standard method GB glucoamylase enzyme activity assay preparations used in Fig. 6.

From the Fig. 6, the difference of measured values in Fig. 4 between extraction temperature 40°C and the extraction temperature 30°C is not great and the same 2 g sample volume measured by the amount of sample values greater than 5 g measured value.

In summary, the national standard method measured rice-koji glucoamylase activity, the sample size has some influence on the experimental results, small amount of sample, the measured value greater. In addition, the extract dilution assay greater impact, but extraction temperature 40°C and 30°C little difference between the measured values.

Comparison of determination methods using the same extracted enzyme:

Enzyme extracted by white spirit koji method: White spirit koji glucoamylase extraction saccharification liquid immersion Timmy song, different measurement methods glucoamylase activity measured values (Table 1).

From Table 1, we can see, same extraction temperature 30°C, rice-koji glucoamylase GB without dilution assay, sample volume impact, glucoamylase activity measured values remain unchanged; DNS assay, Fehling assay at the same dilution, sample volume is not large impact on the results. However, in the same sample volume, diluted 20-fold DNS method about twice the value measured when diluted 10-fold, 10-fold dilution of the measured values of Fehling assay time of about four times the 20-fold dilution; Same 20-fold dilution, rice-koji glucoamylase from GB dilution assay sample volume impact, glucoamylase activity measured values remain unchanged, DNS assay, Fehling assay under the same extraction temperature, sample volume effect on the results is not great, however, when the same amount of sample, 30°C extraction of DNS law, Fehling assay measured values were greater than 40°C extraction measured values. In summary, rice-koji glucoamylase activity was determined by leaching when glucoamylase extraction white spirit in koji, several measurement methods, GB measured rice-koji glucoamylase without dilution, extraction temperature and sample volume. The DNS method, determination of the law affected Fehling dilution and extraction temperature and by the dilution factor greater impact. It also can be seen from the table, the value of the result is different using different measuring method.

Table 1: The glucoamylase enzyme activity of extraction method by white spirit koji glucoamylase

Dilution/ Items	Extraction solution diluted 10 times 30°C			Extraction solution diluted 20 times 30°C			Extraction solution diluted 20 times 40°C		
Method	GB	DNS	Fehling	GB	DNS	Fehling	GB	DNS	Fehling
2gdry koji	57.99	27.55	200	57.99	56.91	56	57.99	49.71	36
5gdry koji	57.99	26.96	170	57.99	53.31	64	57.99	48.76	40

GB, DNS, Fehling glucoamylase enzymes were measured in GB glucoamylase enzyme assay; soy sauce rice-koji glucoamylase activity was measured in the DNS assay; white spirit koji glucoamylase activity assay of Fehling reagent assay; Table values are in u/g

Table 2: The glucoamylase enzyme activity of extraction method by the glucoamylase of GB enzyme preparations

Dilution/ Items	Extraction solution diluted 10 times 30°C			Extraction solution diluted 20 times 30°C			Extraction solution diluted 20 times 40°C		
Method	GB	DNS	Fehling	GB	DNS	Fehling	GB	DNS	Fehling
2gdry koji	318.95	156.67	180	202.97	137.91	396	231.96	86.31	80
5gdry koji	231.96	26.96	160	188.47	146.11	60	144.98	75.51	80

Table 3: The glucoamylase enzyme activity of extraction method by sake koji glucoamylase enzyme

Extraction/Items	White spirit koji glucoamylase extraction	Sake koji glucoamylase extraction			
Method	GB	GB	DNS	Fehling	Kit
2gdry koji	173.97	376.94	634.38	100	417.39
5gdry koji	173.97	173.97	748.19	100	392.04

Enzyme extracted by GB method: From Table 2, we can see, same extraction temperature 30°C, when rice-koji glucoamylase GB assay carried out, Fehling law uses the same dilution; 2 g sample volumes measured by the amount of sample values are all greater than 5 g measured value. The DNS method diluted 20-fold in the measured values 2 g are greater than 5 g sample volume measured amount of sample values and the DNS method both in the diluted 10-fold the greatest difference, but Fehling method when diluted 20-fold difference greater; at the same amount of sample, diluted 10-fold GB method measured values of the measured value is greater than 20-fold, DNS assay, Fehling assay did not rule; The same 20-fold dilution, rice-koji glucoamylase GB assay extraction at the same temperature, 2 g sample volume measured values were greater than 5 g sample volume measured values, DNS France affected by the sample size is not large, Fehling assay extraction during 30°C 2 g sample is much larger than the measured value of the 5 g measured quantity value, but not at 40°C extraction time and sample volume affected; in the same sample amount, DNS extraction method at 30°C measured value is greater than 40°C, GB law, Fehling law have no rule. In summary, rice rice-koji glucoamylase activity was determined by extraction of GB glucoamylase enzyme preparations, the results of several measuring methods all change as sample volume, dilution, extraction temperature change.

Compare of assay results between white spirit extraction, extraction method and Japanese sake extraction method:

Compare of assay results between white spirit extraction and Japanise sake extraction method: From the above experimental results, rice-koji glucoamylase activity assay, using white spirit koji glucoamylase extraction extract, using glucoamylase activity of enzymes national standard measurement method is a combination of measuring method more suitable. Japanese sake brewing uses rice-koji, now compare the extraction and method of sake koji glucoamylase enzyme, Results are as follows, glucoamylase activity measured values are shown in Table 3.

As the design principle of the kit is to exclude the influence of other impurities, such as α-amylase, glucose sample itself, so no need dialysis can be directly measured, however, these results can be seen, Fehling reagent measured results are very stable, Therefore, to verify whether the law applies to Fehling reagent Sake koji glucoamylase extraction method, again by dialysis comparison glucoamylase activity was measured value.

Effect of dialysis on the activity analysis results of Japanese sake: Weigh 2 g curvature (absolute dry weight) respectively, containing 0.5% NaCl was added 10 mL of 10 mM acetate buffer (pH5.0) in the leaching, refrigerator at 4°C overnight extraction, filter paper, the filtrate 10 mL, into the dialysis bag, in the M/100 acetic acid buffer and dialyzed overnight and then washed with distilled water and the dialysate volume to 20 mL (note that the dialysis bag with a little water), this solution is the enzyme solution.

Weigh 5 g curvature (absolute dry weight) respectively, containing 0.5% NaCl was added 25 mL of 10 mM acetate buffer (pH 5.0) in the leaching, refrigerator at 4°C overnight extraction, filter paper, the filtrate 10 mL, into the dialysis bag, in the M/100 acetic acid buffer and dialyzed overnight and then washed with distilled water and the dialysate volume to 20 mL (note that the dialysis bag with a little water), this solution is the enzyme solution. The results are shown in Table 4.

Table 4: The glucoamylase enzyme activity of extraction method by sake koji glucoamylase enzyme (dialysis)

Extraction/Items	Sake koji glucoamylase enzyme (no dialysis)				Sake koji glucoamylase enzyme (dialysis)			
Method	GB	DNS	Fehling	Kit	GB	DNS	Fehling	Kit
2 g dry koji	376.94	634.38	100	417.39	29	558.19	200	419
5 g dry koji	173.97	748.19	100	392.04	43.49	418.57	204	399.21

From Table 3 and 4, it is indicated that while Glucoamylase of koji in white spirit is leached, national standard method measuring results shall be used and there is a big gap in table; the reason may be that it is a group of freshly rice-koji and the song immediately after the measurement, but in Table 1 and 2, koji for different batches of koji (refrigerator); Use sake rice-koji glucoamylase extraction, in several measurement methods, the sample size is not affected in Fehling law, but affected by hemodialysis; in GB and France, 2 g sample method in the measured value is greater than the amount of 5 g sample volume measured values, the difference greater in former, but little difference value is in latter one; Dialysis or not the measured value is little different, which is also consistent with the principles set for kit, but greater deviations is found in Fehling reagent. In summary, rice-koji glucoamylase activity was measured using sake rice-koji glucoamylase extraction, between several measurement methods, France is the most stable method, GB, DNS law by the amount of sample, dialysis or not affected, especially GB, Fehling law not affected by sample size but affected by dialysis, also in comparison sake assay kit shows that the measured values and their domestic measurement values quite different in several ways.

As showed above, we can see that when using liquor koji glucoamylase extraction, several measured values were small than that of the other two methods. And in this extraction method, national standard method is the most stable; the measured value does not vary with sample amount, dilution, extraction temperature. But GB assay for GB enzyme glucoamylase extraction and soak Aspergillus glucoamylase extraction was not applicable, the reason may be that the leaching solution or pH was different, proposing certain other substances interfered with the determination; When using the national standard in enzyme glucoamylase extraction, the measured values were greater than that of liquor koji glucoamylase extraction and several measured values were unstable with the sample amount, dilution, extraction temperature; When using sake Aspergillus glucoamylase extraction method, the measured values of several measurement methods were too large, the more stable measurement method was the kit method, kit method was to exclude the other impurities, such as α- amylase, affected glucose sample itself, so the measured value was stable, measurement operation was simple and quick, but the kit was imported reagents, measurement costs was high. Fehling law was not affected by the sample amount, but by the dialysis or not and it was very unstable in the other two extraction methods, especially liquor rice-koji glucoamylase extraction method, GB assay and DNS were affected by the sample amount; When Aspergillus glucoamylase activity was measured using liquor koji glucoamylase extraction, extract solution was the buffer and a certain amount of water, so the ratio of buffer and water might reach saturation to glucoamylase extraction, perhaps certain glucoamylase can not be leached out in this leach white spirit ratio, so the national standard measurement results were small but steady; DNS was measured by colorimetric method, the polysaccharide sample was not a single sample after the reaction, but several species were mixed, therefore the selected wavelength was not under the maximum absorption, resulting in the measured values were unstable; Fehling reagent was with long measurement time and of many impact factors (Cao, 2001); When Aspergillus glucoamylase activity was measured using the national standard enzyme glucoamylase extraction method, extraction solution was totally buffers, extraction was more thoroughly, the soaked enzyme included glucose amylase and also α- and α-glucosidase enzyme (Wu and Li, 1996). α- amylase activity does not affect the glucoamylase activity and has a catalytic role that glucoamylase will be converted to glucose, it is precisely because of its catalytic role and the measurement results of glucoamylase were high (Brewing Society of Japan, 1993; Wu and Li, 1996); When Aspergillus glucoamylase activity was measured using method, because pH value (pH = 5.0) of sake koji glucoamylase extraction solution was different from that of several other methods (pH = 4.6), the measured values were not the same.

CONCLUSION

In conclusion, the measured results of different measurement methods were quite different, liquor koji glucoamylase measurement results was 36~200 u/g rice-koji; national standard enzyme assay results of glucoamylase activity was 144.98~318.95 u/g rice-koji; DSN method result was 26.96~146.67 u/g rice-koji; sake glucoamylase activity assay result was 392.04~419 u/g rice-koji; The sample amount for GB glucoamylase enzyme preparation was particularly evident, enzyme extracted at 30°C were greater than that measured at 40°C. Extract dilution had greater impact on the experimental results than extraction temperature; Results of different extraction methods with same measurement method show that, the activity with rice-koji glucoamylase extraction method in sake was greater than that of the other two extraction methods; Results of different measurement methods

with same extraction method show that, measurement methods in several assays had some adaptability in different extraction methods. In short, rice-koji glucoamylase activity in sake was the most stable, best embodying the rice-koji glucoamylase activity. Rice-koji glucoamylase in sake extraction method (by dialysis) with DNS measurement method, the results were relatively stable, closest to it. Aspergillus glucoamylase activity measured by white spirit extraction with national standard assay, results were more stable, but were larger than sake Aspergillus glucoamylase activity measured results.

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