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Optimization of malting and mashing conditions of amaranthus grains as a brewing source

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Abstract
Amaranthus grains have high starch content which can be converted to sugars by malting and mashing processes. 'Suvarna' variety of amaranthus was screened for it's amylase activity and reducing sugars. Grains were soaked at three different time intervals of 8 h, 12 h and 16 h and germinated subsequently for 2 and 3 days. The highest amylase activity (850.19 protein/mg/min) was recorded for amaranthus grains soaked for a time interval of 16 h and germinated for 3 days. Similarly, grains recorded the highest reducing sugars (19.71 mg/g) at a soaking time of 16 h and a germination period of 3 days. Commercial α-amylase was used at different concentrations of 0.1, 0.5 and 1 percent at different incubation temperature and incubation period to release of maximum reducing sugars. The highest reducing sugars (91.40 mg/g) were recorded at an enzyme concentration of 1 percent at 65 °C for an incubation period of 24 h. It was also observed that the reducing sugars is inversely related to inoculums levels and yeast strain *Saccharomyces cerevisiae* NCIM3391 showed high alcohol content at 2 percent (5.450%). Alcohol content increases with increase in the fermentation days and decrease in reducing sugars. Whereas with increase in the fermentation days the PH of the beer decreases with decrease in tannin content.

Keywords: Amaranthus, malting, mashing, brewing, alcohol

Introduction
Amaranth (*Amaranthus* spp.,) originates in South America. Botanically, amaranth belongs to the family of dicotyledonous plants Amaranthaceae, which includes more than 60 species, most of which are weeds. Amaranth is richer in proteins and lipids, compared to common cereals. Amaranth grain is rich in phytochemicals (secondary metabolites), such as routine, nictoflorin and isoquercetin, which have a positive effect on human health. It does not contain gluten, so it can be used in the diet of celiacs. Also, the plant is resistant, adaptable and easy for breeding and growing. The color of the grain varies from milky white to yellow, gold, red, brown and black, which depends on the content of the betalaine pigments. Amaranth grains are very small, 1-1.5 mm in diameter, lens shaped and weigh about 0.6-1.3 mg per grain.
Amaranth flour can be used in the production of various types of breads, such as chapatti and tortilla. Amaranth grain can be popped, similarly to the grain of corn. In addition to the common application of barley malt, beer can also be produced from amaranth. Since amaranth grains contain relatively large starch content, strong alcoholic beverages can also be produced. Hence, amaranthus could be a new potential substitute for barley and could raise economic benefits. Thus, the present investigation lies in optimising different parameters for malting and mashing of amaranthus grains.

Materials and Methods
The amaranthus variety selected for testing the suitability for beer production was 'Suvarna' which was procured from GKVK, Bangalore. Initial content of starch was estimated by Anthrone method (Hodge and Hofreiter, 1962) [2] and the reducing sugars were estimated by 3, 5-dinitrosalicylic acid method (Miller, 1959) [3]. The grains were soaked at different time interval of 8h, 12h and 16h in normal potable water and subsequently germinated at two different periods of 2 days and 3 days in clean muslin cloth. Amylase activity and reducing sugars were determined. The malt was kilned at 60 °C in for 8h. The kilned grains were further broken into grits by running through a blender at low speed. Mashing was carried out with grist to water ratio of 1:4 along with commercial α-amylase (Himedia). The enzyme concentrations of 0.1, 0.5 and 1 percent were tested for saccharification of the substrate at two temperature levels of 45 °C and 65 °C and different incubation periods of 8, 16 and 24 h were

used for optimization of mashing parameters.

The optimized parameters for malting and mashing were used for wort preparation. The wort obtained after double filtering the mash, was boiled for an hour. For flavouring, hops in the form of pellets (Acid extracted, Northern Brewer Hops) was added @ 40 ppm after 30 minutes of boiling. The pH, total soluble solids (TSS), tannin content and reducing sugars were estimated.

Amylase activity was estimated by the method described by Bernfield (1955) [1]. Reducing sugars were determined by 3, 5-dinitrosalicylic acid method (Miller, 1959) [3]. The pH meter of Analog model was used to record the pH of wort. Total soluble solids (TSS) was determined with the help of ERMA hand refractometer having range of 0-32 °Brix at 20 °C. Folin-Denis method was used for estimation of tannins (Schanderl, 1970) [5]. The experimental results were statistically analyzed as per Fisher's analysis of variance technique (Panse and Sukhatme, 1961) [4].

Results and Discussion

Estimation of starch and reducing sugars content of amaranth grains for beer production

Amaranth grains were analysed for their efficiency based on reducing sugars and starch content of grains. The initial contents of starch of amaranth grains was found to be 49.5% and initial reducing sugars content was found to be 0.45% (Table 1).

Table 1: Amount of starch and reducing sugars present in amaranthus seeds (Suvarna variety)

Sl. No.	Parameter	Quantity (%)
1	Starch	49.5
2	Reducing sugars	0.45

Effect of period of soaking and germination of amaranth on amylase activity

Among germination periods significantly higher α-amylase activity (772.88 µg of protein/mg/min) was observed on the third day of germination. Grains germinated for two days showed lower amylase activity (644.73 µg of protein/mg/min).

Among three soaking periods, 16 h of soaking showed the highest α-amylase activity (761.53 µg of protein/mg/min) which was significantly greater than 12 h of soaking period (715.27 µg protein/mg/min). Least enzyme activity was observed with 8 h of soaking which was found to be 649.63 µg protein/mg/min (Table 2).

Effect of period of soaking and germination of amaranth on reducing sugars

Among germination periods significantly higher amount of reducing sugars (17.27 mg/g) was observed on the third day of germination. Grains germinated for two days showed lower content of reducing sugars (13.21 mg/g). Among three soaking periods, 16 h of soaking showed the highest content of reducing sugars (17.69 mg/g) which was significantly greater than 12 h of soaking period (15.79 mg/g). Least content of reducing sugars was observed with soaking period of 8 h (12.22 mg/g) of germination (Table 2). The interaction effect between soaking period and germination period were found to be significant.

Table 2: Effect of period of soaking and germination of amaranthus on amylase activity and reducing sugars

Sl. No.	Germination days (A)	Amylase activity (µg of protein/mg/min)				Reducing sugar (mg/g)			
		Soaking period (B)							
		8 h	12 h	16 h	Mean	8 h	12 h	16 h	Mean
1	2 days	605.833	655.500	672.867	644.73	10.313	13.640	15.677	13.210
2	3 days	693.420	775.047	850.193	772.88	14.133	17.950	19.713	17.266
Mean		649.63	715.273	761.530	708.81	12.223	15.795	17.695	15.24
Source		SEm±		CD at 1%		SEm±		CD at 1%	
Germination days (A)		0.985		3.55		0.117		0.567	
Soaking period (B)		0.207		4.35		0.143		0.694	
Interaction (AXB)		0.707		6.16		0.203		0.982	

Table 3: Optimization of incubation temperature, incubation period and α-amylase concentration for mashing

Concentration of enzyme	45 °C				65 °C			
	Period of incubation (h)							
	8 h	16 h	24 h	Mean	8 h	16 h	24 h	Mean
	Reducing sugars (mg/g)							
0.1%	27.855	37.850	32.03	32.58	33.250	54.300	59.65	49.06
0.5%	35.150	40.900	37.35	37.8	49.745	69.985	75.87	65.2
1.0%	44.015	51.400	46.35	47.26	66.600	86.180	91.40	81.39
Mean	35.69	43.38	38.58	39.22	49.87	70.16	75.64	65.22
Source	SEm±				CD at 1%			
Incubation temperature(A)	0.200				0.672			
Incubation period (B)	0.245				0.823			
Enzyme concentration(C)	0.245				0.823			
AB	0.346				1.164			
BC	0.424				1.425			
AC	0.346				1.164			
ABC	0.599				2.02			

Effect of commercial α-amylase enzyme on hydrolysis of starch in amaranth grains

Commercial α-amylase enzyme (Himedia) was added during mashing process to promote hydrolysis of starch from grains. Standardization of commercial enzyme concentration, incubation temperature and incubation period was done. The results obtained are presented in Table 3.

Optimization of concentration of α-amylase enzyme, incubation temperature and incubation period for mashing.

The results clearly showed that the substrate, which was subjected to commercial α-amylase enzyme at three different concentrations showed a positive effect towards releasing of fermentable sugars from a lower concentration of enzyme to higher concentration. Among three different concentrations of α-amylase, the grains treated with 1% of enzyme released the highest reducing sugars (81.39 mg/g) at 65 ºC and least reducing sugars (32.58 mg/g) were recorded at a concentration of 0.1 percent at 45 ºC. At two different temperatures, the highest reducing sugars (65.22 mg/g) were released at 65 ºC temperature and the least reducing sugars (39.22 mg/g) were released at 45 ºC (Table 3).

Among three different incubation periods, significantly higher reducing sugars (75.64mg/g) were released with incubation period of 24 hours at 65 ºC, followed by 16 hours of incubation at 65 ºC (70.16 mg/g). The least reducing sugars (35.69 mg/g) were recorded at 45 ºC with an incubation period of 8 hours (Table 3).

Effect of fermentation period on alcohol content and residual reducing sugars of amaranthus (Suvarna) beer

It was observed that with increases in the fermentation days of beer the alcohol content increases due to increase in amalyse activity and decrease in reducing sugars content in beer it occurs due to the yeast which converts the sugar into the alcohol (Table 4)

Table 4: Effect of fermentation period on alcohol content and residual reducing sugars of amaranthus (Suvarna) beer

No. of days	Alcohol (%)	Residual reducing sugars(mg/g)
5	5.48	20.46
7	5.78	18.16
10	6.15	15.51
Mean	5.80	18.04
C.D.	0.103	0.713
SE(m)	0.029	0.202
C.V.	0.87	1.93

Effect of period of fermentation on pH and tannin content of amaranthus (Suvarna)

It was observed that with the increase in number of fermentation days there was decrease in pH and tannin content of beer (Table 5).

Table 5: Effect of period of fermentation on pH and tannin content of amaranthus (Suvarna)

Period of fermentation (days)	pH	Tannin content (mg/100 ml)
5	4.92	5.11
7	4.62	4.79
10	4.22	3.91
mean	4.59	4.6
C.D.	0.046	0.096
SE(m)	0.013	0.027
C.V.	0.498	1.02

Properties of wort prepared on bench scale from amaranth grains TSS, pH, reducing sugars and tannin content

The pH of wort prepared from amaranth was 6.2. The tannin content was found to be 7.05 mg/100 ml. TSS of wort was 5ºBrix. The reducing sugars were recorded to be 92.5 mg/g (Table 6).

Table 6: Properties of wort prepared on bench scale from amaranthus variety (Suvarna) for beer production

Sl. No.	Parameters	Values
1.	pH	6.2
2.	Total Soluble Solids (TSS)	5 °B
3.	Tannin Content	7.05 mg/100 ml
4.	Reducing Sugars	92.5 mg/g

Sensory Analysis of Amaranthus beer with commercial beer

Organoleptic evaluation of Amaranthus beer and commercial beer is carried out using 16 – point Hedonic Scale and the beer is found to be acceptable with 13.25 score compared to commercial beer i.e., 14.75 (Table 7)

Table 7: Organoleptic evaluation of amaranthus beer in comparison with commercial beer

Sl. No.	Characteristics	Total Score	Average score given by panel judges based on 16 point scale	
			Commercial beer (A)	Amaranthus beer (B)
1.	Appearance	2	2.00	2.00
2.	Colour	2	1.50	2.00
3.	Aroma	2	1.75	1.25
4.	Total acidity	2	1.50	1.50
5.	Body	2	2.00	1.75
6.	Flavour	2	1.75	1.50
7.	Astringency	2	1.75	1.50
8.	General quality	2	2.00	1.75
9.	Overall acceptability	2	14.75	13.25

Conclusion

It was observed that the *Saccharomyces cerevisiae* NCIM3391 showed high alcohol content at 2 percent and it also observed that with increase in fermentation days pH & Tannin content of the beer decreases and the overall acceptability increases with ageing of the beer.

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