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RESEARCH ARTICLE

Studies on Potato (*Solanum tuberosum* L.) cultivars for Ethanol Production.

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Abstract

Five potato cultivars evaluated for proximate characteristics of dry matter, moisture content, starch, total sugars and reducing sugars presented significant differences. Kufri Pukhraj with the highest amount of reducing and total sugars was selected for saccharification. A comparison of liquefaction and saccharification carried out using crude α-amylase (barley malt) followed by crude glucoamylase (*Aspergillus flavus*) and by using commercial amylases (α-amylase and glucoamylase) showed a maximum of 3º and 8ºB brix with crude and commercial amylases, respectively.

Introduction:-

Potato (*Solanum tuberosum* L.) is one of the principal important tuberous food crops in the world, either for direct consumption or processing as it contains 16% carbohydrates, 2% protein, 1% minerals, 0.6% dietary fibre and negligible amount of fat (Ramezani and Aminlari, 2004). Potatoes also contain significant levels of phenolic compounds and vitamin C as potent antioxidants (Brown, 2005). The latter are known to inactivate reactive oxygen species, reduce oxidative damage, improve immune functions and reduce risk of cardiovascular diseases, cancer, cataract, diabetes and aging (Kaur *et al*., 2012).

In Punjab, among vegetable crops, about half of the total area is covered under potato with an area of about 87.2 thousand ha and production of 2189.2 thousand tonnes (Saxena and Gandhi, 2015). The quest of potato processors for fresh potatoes with desirable processing attributes (high dry matter and low reducing sugars) is unending. But 70% of the potato produce belongs to non processing varieties that result in glut like situation every year which is further aggravated by shortage of storage facilities thus leading to low remuneration prices. Both these problems may be solved if an alternative use of potato is found.

Potatoes being rich in starch make it an ideal substrate for ethanol production. The good quality ethanol produced from potatoes can be used both for fuel as well as potable purposes (Khan *et al*., 2012). Therefore, the aim of this study was to evaluate the available varieties in the region particularly non-processing ones for and their suitability with respect to production of fermentable sugars that may be fermented to ethanol.

Materials and methods:-

Well cured, healthy tubers of five potato cultivars var. Kufri Chandramukhi, Kufri Jyoti, Kufri Pukhraj, FL 1533 and Kufri Badshah were procured from Department of Vegetable Science, PAU, Ludhiana.

Proximate analysis:-

The proximate analysis of potato varieties included the estimation of dry matter/moisture content (AOAC, 2002), starch content (Clegg, 1956), total sugars (Dubois *et al*., 1956) and reducing sugars (Miller, 1959).

Hydrolysis of Starch:-

Potato was gelatinized prior to enzymatic hydrolysis as it is very important since enzymes are only active either on gelatinized or mechanically damaged starch granules (Yekta and Ulgar, 1994). For the purpose, potato slurry was heated to 100ºC and allowed to stand for 30 minutes for gelatinization. Enzymatic hydrolysis of pre-gelatinized potato mash was accomplished by treatment employing commercial amylases i.e., α-amylase and glucoamylase (Novozymes, India) and a combined treatment with barley malt and crude amylase enzyme produced by *Aspergillus flavus* MTCC 8233 but prior to that gelatinization of potato starch was done. The latter involved addition of 20g barley malt (prepared by germinating barley seeds in trays at 28ºC) to 100 ml of gelatinized potato and heating the mix to 80ºC for 60 min. The crude glucoamylase enzyme (10 ml) produced from *Aspergillus flavus* MTCC 8233 (100 rpm, 30ºC for 96h) for 3 hours. Similar conditions were employed with commercial α-amylase (200KNU/ml) and glucoamylase (400 AGU/ml). The obtained ºbrix was recorded by brix Erma hand refractometer.

Results and discussion:-

Proximate Characteristics of Potato Varieties:-

Five varieties of potato (*Solanum tuberosum* L.) viz. Kufri Badshah, Kufri Chandarmukhi, Kufri Jyoti, Kufri Pukhraj and FL 1533 were subjected to proximate analysis in terms of moisture content, dry matter, starch, total sugars and reducing sugars. Among these varieties, Kufri Chandarmukhi and Kufri Jyoti has been recommended for processing into instant flakes and chips whereas other varieties are not recommended for processing (Singh *et al.*, 2009). It was also observed that starch content was maximum in Kufri Chandarmukhi i.e. 17.1% and minimum in Kufri Badshah 12.74% (Table 1). On dry matter basis, these varieties were observed to contain 60 to 74% starch matter. Dry matter of these five potato cultivars was observed between 17-24% with maximum in Kufri Chandarmukhi (23.22%) and minimum in Kufri Pukhraj (17.99%). In literature, it has been reported that dry matter content of potato affects starch hydrolysis as potatoes with high dry matter content are difficult to process for starch hydrolysis (Liimatainen *et al*., 2004).

Total sugars could also serve as quality index for ethanol production. Large variations in total sugars were observed among different cultivars. Total sugars were found to be maximum in cultivar Kufri Pukhraj (0.81% on wet basis and 4.50% on dry weight basis) and lowest in 1533 variety (0.35% on wet and 1.59% on dry weight basis). Literature also reports that among eleven different Indian potato cultivars the maximum total sugar was found in Kufri Pukhraj (0.77%), making it unsuitable for processing (Kaur and Aggarwal, 2014). This is because reducing sugars induce a non-enzymatic Millard (browning) reaction with free amino acids during frying, resulting in desirable color and flavor of fried potato products which also affects consumer acceptability (Kaur *et al*., 2012). Thus varieties with high sugar aren't suitable for processing into chips and other starch food products. In this study, reducing sugars ranged 0.20-0.48% on wet weight basis among different cultivars, with maximum in Kufri Pukhraj (0.48% on wet and 2.67% on dry weight basis) and lowest in FL 1533 (0.20% on wet and 0.91% on dry weight basis). The total and reducing sugars were maximum in Kufri Pukhraj thus making it unsuitable for processing. However, these high reducing sugars are desirable for ethanolic fermentation. Hence in the present study, Kufri Pukhraj was selected for its starch hydrolysis.

Hydrolysis of potato mash:-

The hydrolysis (liquefaction and saccharification) of potato mash carried out by using crude α-amylase produced by barley malt followed by 48h old culture of *Aspergillus flavus* and by using commercial amylases (α-amylase and glucoamylase). The results presented in Table 2 revealed an incomplete liquefaction with barley malt where OD_{600} decreased from 0.883 to 0.594 in 3 hours at 80ºC. Accordingly the brix produced by using crude glucoamylase from *Aspergillus flavus* was 3ºB in 3h at 60ºC. On the other hand, with commercial amylases the residual starch OD decreased from 0.693 to 0.210 and the brix level increased from 0 to 8ºB. Therefore, on the basis of brix obtained commercial amylases were selected for starch hydrolysis. As the malted barley cleaves only α-1, 4-linkages from non-reducing ends, to give limit dextrins and β-maltose which results in incomplete liquefaction (Atnafu and Abebaw, 2015) and the maximum amount of enzyme production by *Aspergillus flavus* was obtained with 62% moisture content (Geetha *et al*., 2011).

The study thus identified Kufri Pukhraj as a suitable cultivar for enzymatic hydrolysis into fermentable sugars that can be fermented to produce ethanol. The trials in this regard are being carried out in our laboratory.

Table 1: Physiochemical Analysis of Potato Varieties.

Parameters (%)	Varieties					CD (5%)
	Kufri Badshah	Kufri Chandarmukhi	Kufri Jyoti	1533	Kufri Pukhraj	
Moisture content	79	76.78	81.30	77.95	82.01	1.843
Dry matter	21	23.22	18.7	22.05	17.99	0.268
Starch	12.74	17.10	13.40	14.07	13.00	0.354
Total sugar	0.60	0.36	0.65	0.35	0.81	0.381
Reducing sugar	0.26	0.21	0.40	0.20	0.48	0.422

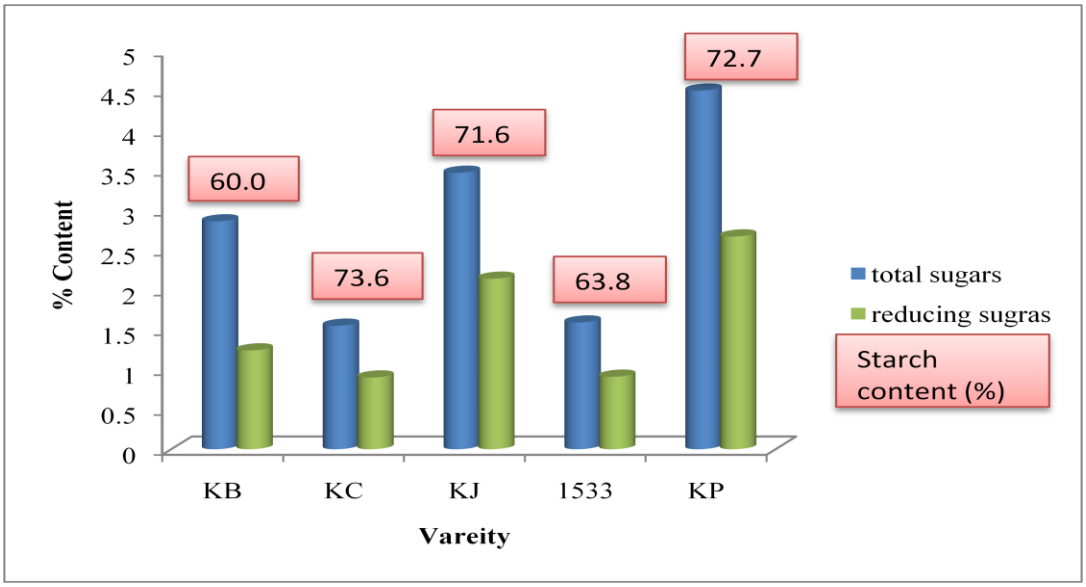

Fig 1: Chemical composition (percent) on dry weight basis in different varieties of potato

Table 2: Comparative starch hydrolysis using barley malt-*Aspergillus flavus* MTCC 8233 glucoamylase and commercial amylases.

Time (h)	Crude enzyme		Commercial amylases	
	Barley malt	*Aspergillus flavus* glucoamylase	α-amylase	Glucoamylosidase
	Residual starch (OD_{600})	Brix (°B)	Residual starch (OD_{600})	Brix (°B)
0	0.883	0	0.693	0
1	0.801	0.6	0.469	5
2	0.683	1.9	0.212	8
3	0.594	3	0.210	8

* glucoamylases were added after 3h of malt/α-amylases liquefaction

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