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## To study the storage stability of mango wine during different conditions with pretreatment

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**Abstract**

In this research, Fermentation was carried out in conical flasks at room temperature for 15 days. The flasks were closed using a cotton plug and polyethene with a bend of thread. Fermentation rate was monitored every 24 hours by checking the change in TSS (°Bx). End of fermentation was determined when the TSS could not change any further. After fermentation, the wine samples were filtered (vacuum filter using .2µm filter paper) prior to analysis. All the determinations were done in triplicates and the mean values determined. The clear supernatant samples were kept in refrigerator for a few weeks until the physicochemical and sensory analysis were completed. At the end of fermentation, the wines were stabilized with the addition of KMS @ 85 ppm and preserved. After 30 days the highest score awarded for overall acceptability was 7.67 in case of Chausa wine sample having 10% inoculum concentration and IARI strain. Whereas, the lowest score was observed as 7.07 in the wine sample of Gulab jamun and Dasheheri variety having 15% inoculum concentration with both strains. But after 45 days the highest score awarded for overall acceptability was 7.52 in the wine sample of Chausa variety having 5% inoculum concentration with native strain. Whereas, the lowest score was awarded as 7.05 in the wine sample of Gulab jamun variety having 15% inoculum concentration with native strains. The highest score for overall acceptability was awarded as 7.27 in the wine sample of Chausa variety having 5% inoculum concentration with IARI strain. Whereas, the lowest score was 6.92 in the wine sample of Gulab jamun variety having 10% inoculum concentration with IARI strain after 60 days.

**Keywords:** Storage stability, mango wine, TSS

**Introduction**

Mango is one of the most highly priced desert fruits of the tropics. It has rich luscious, aromatic flavour and a delicious taste in which sweetness and acidity is delightfully blended. Mango production has experienced continuous growth in the last decades of the 20<sup>th</sup> century [2]. The world's total annual mango fruit production was estimated at 22 million metric tonnes. Global production of mangoes is concentrated mainly in Asia and more precisely in India that produced 12 million metric tonnes per annum. Major mango producing countries are India, Mexico, China and Pakistan [4]. Mango is called as the king of fruits and pride fruit of India. In India, mango is grown in 10.85 million hectare and it occupied 39% of total fruit production. More than 25 cultivars of mango are cultivated commercially in various regions of India. Wine making is one of the most ancient technologies and is now one of the most commercially prosperous biotechnological processes. Even though the grapes are the main raw material used for the wine production, there is an increasing interest in the search for indigenous fruits such as orange, apple, mango, and also palm sap that are cheap and readily available for wine making in such countries where grapes are not abundantly available [11]. However the production of wine from mango, which has a high carbohydrate content (16–18% w/v), is one of the alternative ways to exploit and convert the surplus production into a valuable product [5], and it has been proved that mango wine contains bioactive molecules which impart antioxidant activity to the wine [14]. In the processing of mango, peel is a major by-product and represents a serious disposal problem. The use of mango peels for the production of biogas and dietary fiber has been described; however, the studies on peels are scarce. Their use as animal feed is known, although they can also be used for obtaining more valuable products like good quality pectin's [6, 8]. Mango peel is rich in dietary fiber, antioxidant phytochemicals such as carotenoids, polyphenols, anthocyanins, and volatile compounds [1]. It is a safe and inexpensive material, comprising an interesting new support for cell immobilization for wine

fermentation. The preparation of wine or any other beverage using cells entrapped in mango peel has not been attempted yet, and it is a very attractive proposition because of its full compatibility in the wine production. Therefore, the aim of the present study was to investigate the suitability of immobilized cells entrapped in mango peels for mango wine fermentation at various temperatures, as well as the influence of the immobilized biocatalyst on the volatile composition of the produced wines. There has been limited information in the research on mango wine until recently, although it started from 1960's. reported the first study on mango wine production. [7] screened twenty varieties of mangoes from India for wine production. [11] developed a method of mango juice extraction with pectinase and characterized ethanol and some volatile contents of mango wine. Although there are various studies on the effects of pitching levels of brewer's yeasts on beer fermentation, [13] information concerning the effects of the pitching level of the wine yeast *S. cerevisiae* on wine fermentation is scarce. There is still no complete profiling of chemical properties of mango wine at varying temperatures and yeast inoculum sizes. Although a complete profile of chemical and volatiles of fresh mango juice is available [9, 10]

### Materials and Methods

The present study was undertaken to develop fermented alcoholic beverage (wine) from different varieties of mango in the Department of Agricultural Engineering and Food Technology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut. Studies were also carried out on fermentation of wine to hydrolyze fermentable sugars with different pre-treatment methods, to select different efficient yeast strains and to study the nutritional and biochemical properties of wine prepared from mango varieties. All samples were kept in refrigerator at 5 °C for further analysis.

#### Procedure for Isolation of Genomic DNA (C2)

For genomic DNA isolation CTAB method [3] with some modification was used. CTAB (Cetyl-trimethyl-ammonium bromide) is a cationic detergent, which solubilizes membranes and forms a complex with DNA. For isolation of DNA following steps are as follows.

1. Fresh mycelia (~36h old) was filtered from PDB, washed with 0.9% NaCl solution, squeezed to remove excess water and ground to fine powder with mortar and pestle using liquid nitrogen, 100mg powdered biomass was transferred to the 600µl of pre-warmed (65 °C) extraction buffer in 1.5ml centrifuge tube. The sample was mixed after every 15 min.
2. After 10 min add 50µl 20% SDS. Samples were subjected to 1 hr incubation at 65 °C.
3. After cooling, an equal volume of Chloroform: Isoamylalcohol (24:1) was added to the tube and mixed gently for 15-20 minutes.
4. The tubes were then centrifuged (CPR-24, Remi India, Rotor No. 8) at 4,000 rpm for 05 minute, at 10 °C.
5. After centrifugation aqueous phase was transferred to fresh tube. Added ~0.6 volume of chilled isopropanol and kept at -20 °C for overnight.
6. The tubes were centrifuged at 10,000 rpm for 10 minutes at 4 °C. After centrifugation, supernatant was discarded and pellet was washed with 70% ethanol.

Finally the pellet was air dried in laminar flow and was dissolved in 60 µl of TE buffer according to pellet.

#### Purification of Genomic DNA (C3)

For purification of DNA following steps are as follows.

1. 3.0 µl of RNase (10 mg/ml) was added to 60 µl of DNA solution, and incubated at 37 °C for 1 hour.
2. Equal volume of P: C: I (25:24:1) was added to the sample and mixed gently by inverting the tube.
3. Tube was spin at 10,000 rpm at room temperature for 10 minute.
4. Aqueous layer was collected and equal volume of C: I (24:1) was added to the sample and mixed gently by inverting the tube.
5. Tube was spin at 10,000 rpm at room temperature for 10 minute.
6. Aqueous layer was collected, ~0.6 volume of chilled isopropanol was added and kept at -20 °C for overnight.
7. Then centrifuged at 10,000 rpm for 10 minutes at 4 °C.
8. Discard the supernatant and add 200 µl of 70% ethanol. Mixed gently for 2-4 times and spin with 10,000 rpm for 5 minutes at 4 °C.
9. Discard the supernatant and dry the pellet at 37 °C, dissolve in 30 µl TE buffer and store at 4 °C.

#### Storage of DNA (C4)

DNA was stored at 4 °C, for immediate use in triple distilled autoclaved water and at -20 °C in TE buffer for long term storage.

#### Agarose gel Electrophoresis (C5)

Agarose gel electrophoresis of the isolated genomic DNA was performed in electrophoresis assembly (Genei, midi model) to know the quality of DNA. As the yeast (*Saccharomyces Cerevisiae*) genome size is ~13Mb, a 0.8% gel was used to visualize the genomic DNA, as it can resolve DNA molecules in the range of 0.7 to 8.5 kb. 0.8% (w/v) agarose gel was prepared by suspending 0.8g agarose in 100 ml 1x TAE buffer. 5µl ethidium bromide was added and the gel was casted in a gel tray fixed in a gel caster which was kept on horizontal surface. The isolated genomic DNA samples were mixed with a loading dye (8µl sample + 8µl dye and were electrophoreses on 0.8% agarose gel) in 1X TAE buffer at 5 volt/cm of gel tray for ~2/3 run.

### Result and Discussions

The study was undertaken to develop wine using three varieties of mango (Dashari, Chausa and Gulab Jamun), three inoculum concentration (5%, 10% and 15%), and two different strains (IARI and Native). Qualitative analysis of wine was done during storage period. Must of mangoes were prepared with various combinations of inoculation concentrations and strains. For the evaluation of quality of wine several physico-chemical parameters viz. TSS, pH, acidity, ascorbic acid, ethanol, total phenols, reducing sugar, microbial studies (TPC) and sensory parameters were selected and determined. Wine samples were packed in sterilized conical flasks. Evaluation of quality parameters of wine was carried out for fresh as well stored samples after 0, 15, 30, 45 and 60 days in refrigerator at 10 °C storage condition for further analysis. Further, statistical analysis was done to analyze the data and statistical significance of the different parameters were considered under this study.

### Effect on TSS

The TSS content of mango wine showed a decreasing trend for all the treatments with storage period up to 60 days. The study revealed that TSS of the samples having inoculum concentrations of 5%, 10% and 15% with two different strains namely IARI (*Saccharomyces Cerevisiae* 1035) and native were observed as 20 °Brix in fresh samples. From table 01 it was observed that TSS of all the samples decreased with storage period (15, 30, 45 and 60 days). The TSS values of all the wine samples of Dashari, Chausa and Gulab Jamun having inoculum concentration of 5%, 10% and 15% with IARI (*S. Cerevisiae* 1035) and native strain were observed as 0 °Brix in refrigerator at 10 °C after 60 days of storage period.

### Effect on pH

The pH of mango wine showed a decreasing trend for all the treatments with storage period up to 60 days. The pH of the samples of three different mango varieties having inoculum concentrations of 5%, 10% and 15% with two different strains namely IARI1035 and native were measured as 4.5, respectively in the fresh samples. During storage, it was observed from from table 02 that pH of all the samples were decreased at 0, 15, 30, 45 and 60 days of storage. The study revealed that pH values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 5% inoculum concentration with IARI and native strains after 60 days of storage were observed as 3.80, 3.85, 3.82, 3.78, 3.83 and 3.80 respectively. The pH values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 10% inoculum concentration with IARI and native strains after 60 days of storage were observed as 3.75, 3.77, 3.81, 3.74, 3.77 and 3.80 respectively and for 15% inoculum concentration, pH were observed as 3.71, 3.75, 3.77, 3.69, 3.72 and 3.74 respectively. The decrease in pH may be due to the fact that pH has inverse relationship with acidity which may inferred from the results obtained.

### Effect on Acidity

The acidity of samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) having inoculum concentrations of 5%, 10% and 15% with two different strains (IARI and native) were measured as 0.320, 0.360, 0.392, 0.320, 0.371, 0.400, 0.321, 0.370, 0.392, 0.332, 0.374, 0.400, 0.330, 0.370, 0.402, 0.332, 0.370 and 0.400 respectively in fresh samples. During storage period, it was observed (Table 03) that acidity of all the samples were increased at 0, 15, 30, 45 and 60 days of storage. The study revealed that acidity of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 5% inoculum concentration with IARI and native strains after 60 days of storage period were observed as 1.510, 1.600, 1.940, 1.512, 1.662 and 1.942 respectively. The acidity values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 10% inoculum concentration with IARI and native strains after 60 days of storage period were observed as 1.512, 1.610, 1.942, 1.513, 1.664 and 1.943 respectively and for 15% inoculum concentration acidity were observed as 1.514, 1.612, 1.944, 1.514, 1.666 and 1.944 respectively. The increase in acidity of mango wine may be

due to formation of organic acid by ascorbic acid degradation as well as progressive decrease in protein content. The study also revealed that acidity increased with increased in storage period irrespective of storage conditions.

### Effect on Ascorbic Acid

The ascorbic acid of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) having inoculum concentrations of 5%, 10% and 15% with two different strains namely IARI and native were measured as 34.50, 24.00, 28.35, 34.50, 24.00, 28.35, 34.45, 24.00, 28.35, 34.44, 24.00, 28.35, 34.50, 24.00, 28.35, 34.50, 24.00 and 28.35 respectively in the fresh samples. During storage period, it was observed from table 04 that ascorbic acid of all the samples were decreased at 0, 15, 30, 45 and 60 days of storage period. The study revealed that ascorbic acid values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 5% inoculum concentration with IARI and native strains after 60 days of storage period were observed as 20.60, 11.80, 15.80, 20.60, 11.78 and 15.80 respectively. The ascorbic acid values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 10% inoculum concentration with IARI and native strains after 60 days of storage period were observed as 20.55, 11.76, 15.76, 20.50, 11.76 and 15.80 respectively and for 15% inoculum concentration ascorbic acid were observed as 20.50, 11.74, 15.74, 20.45, 11.74 and 15.74 respectively.

### Effect on TPC

The total plate count of the samples of three different mango varieties Dasheheri, Chausa and Gulab Jamun) having inoculum concentrations of 5%, 10% and 15% with two different strains namely IARI and native were measured as 6.95, 7.06, 7.04, 6.94, 7.05, 7.04, 6.96, 7.05, 7.04, 6.95, 7.06, 7.04, 6.96, 7.07, 7.05, 6.94, 7.05 and 7.05 log10cfu/ml respectively in the fresh samples. During storage period, it was observed from table 05 that total plate count of all the samples were decreased at 0, 15, 30 and 45 days of storage period. The study revealed that total plate count values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 5% inoculum concentration with IARI and native strains up to 45 days of storage period were observed as 0.8, 0.9, 0.85, 0.8, 0.91 and 0.84 log10cfu/ml respectively but after 45 days of storage period were observed as 1.40, 1.45, 1.46, 1.41, 1.46 and 1.45 log10cfu/ml respectively. The total plate count values of the samples of three different mango varieties (Dasheheri, Chausa and Gulab Jamun) containing 10% inoculum concentration with IARI and native strains up to 45 days of storage period were observed as 0.78, 0.88, 0.83, 0.78, 0.90 and 0.83 log10cfu/ml respectively but after 45 days of storage were observed minimum as 1.41, 1.46, 1.47, 1.42, 1.45 and 1.46 log10cfu/ml respectively and for 15% inoculum concentration with IARI and native strains up to 45 days of storage period total plate count were observed as 0.76, 0.86, 0.84, 0.77, 0.88 and 0.82 log10cfu/ml respectively but after 45 days of storage period were observed as 1.43, 1.45, 1.46, 1.43, 1.47 and 1.46 log10cfu/ml respectively.

**Table 1:** Effect of inoculum concentrations, strains and storage period on total soluble solid (TSS) of mango wine samples

Storage Period (Days)	TSS																	
	Inoculum Concentration (5%)						Inoculum Concentration (10%)						Inoculum Concentration (15%)					
	IARI Strain			Native Strain			IARI Strain			Native Strain			IARI Strain			Native Strain		
	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G
0	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
15	15.50	15.00	14.83	15.50	15.00	14.83	13.83	13.33	13.66	14.00	13.50	14.00	12.00	11.66	11.50	11.66	11.50	11.50
30	9.66	9.50	9.25	9.66	9.30	9.33	8.66	8.33	8.33	8.83	8.66	8.83	6.33	6.66	6.00	6.66	7.00	6.66
45	1.16	1.00	1.92	1.16	1.00	1.00	0.66	0.83	0.83	0.66	0.86	0.66	0.33	00.00	00.00	00.00	00.00	00.00
60	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
CD	0.981	0.921	0.789	0.981	0.921	0.789	0.412	0.412	0.583	1.064	0.256	0.789	0.981	0.673	0.921	0.673	0.921	0.476
SE(d)	0.435	0.408	0.350	0.435	0.408	0.350	0.183	0.183	0.258	0.471	0.114	0.350	0.435	0.298	0.408	0.298	0.408	0.211
SE(m)	0.307	0.289	0.247	0.307	0.289	0.247	0.129	0.129	0.183	0.333	0.080	0.247	0.307	0.211	0.289	0.211	0.289	0.149
CV	5.744	5.495	4.740	5.744	5.495	4.740	2.590	2.600	3.691	6.636	1.616	4.960	6.883	4.763	6.667	4.763	6.494	3.383

**Table 2:** Effect of inoculum concentrations, strains and storage period on pH of mango wine samples

Storage Period (Days)	pH																	
	Inoculum Concentration (5%)						Inoculum Concentration (10%)						Inoculum Concentration (15%)					
	IARI Strain			Native Strain			IARI Strain			Native Strain			IARI Strain			Native Strain		
	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G
0	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
15	4.35	4.34	4.34	4.34	4.35	4.34	4.29	4.31	4.29	4.32	4.32	4.30	4.31	4.31	4.30	4.30	4.30	4.31
30	4.20	4.21	4.21	4.22	4.20	4.21	4.19	4.17	4.19	4.16	4.17	4.18	4.16	4.16	4.17	4.16	4.15	4.15
45	4.00	4.05	4.04	4.02	4.04	4.03	3.99	4.03	4.00	4.01	4.03	4.02	4.00	4.01	4.00	3.98	3.98	3.99
60	3.80	3.85	3.82	3.78	3.83	3.80	3.75	3.77	3.81	3.74	3.77	3.80	3.71	3.75	3.77	3.69	3.72	3.74
CD	0.012	0.015	0.013	0.013	0.013	0.010	0.014	0.013	0.012	0.010	0.013	0.012	0.012	0.012	0.015	0.012	0.010	0.013
SE(d)	0.005	0.007	0.006	0.006	0.006	0.004	0.006	0.006	0.005	0.004	0.006	0.005	0.005	0.005	0.007	0.005	0.004	0.006
SE(m)	0.004	0.005	0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.004	0.005	0.004	0.003	0.004
CV	0.152	0.195	0.175	0.175	0.174	0.124	0.187	0.176	0.152	0.124	0.176	0.152	0.153	0.152	0.197	0.153	0.125	0.176

**Table 3:** Effect of inoculum concentrations, strains and storage period on acidity of mango wine samples

Storage Period (Days)	Acidity (%)																	
	Inoculum Concentration (5%)						Inoculum Concentration (10%)						Inoculum Concentration (15%)					
	IARI Strain			Native Strain			IARI Strain			Native Strain			IARI Strain			Native Strain		
	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G
0	0.320	0.360	0.392	0.320	0.371	0.400	0.321	0.370	0.392	0.332	0.374	0.400	0.330	0.370	0.402	0.332	0.370	0.400
15	0.580	0.622	0.700	0.580	0.600	0.700	0.590	0.624	0.700	0.582	0.610	0.710	0.595	0.625	0.700	0.584	0.612	0.712
30	0.800	0.862	0.890	0.800	0.861	0.892	0.810	0.864	0.892	0.802	0.862	0.894	0.812	0.864	0.892	0.804	0.864	0.895
45	1.110	1.260	1.640	1.112	1.260	1.642	1.112	1.262	1.642	1.112	1.261	1.642	1.113	1.264	1.645	1.114	1.266	1.644
60	1.510	1.600	1.940	1.512	1.662	1.942	1.512	1.610	1.942	1.513	1.664	1.943	1.514	1.612	1.944	1.514	1.666	1.944
CD	0.004	0.004	0.004	0.004	0.003	0.003	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.003	0.004	0.003	0.005	0.003
SE(d)	0.002	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.001
SE(m)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001
CV	0.245	0.218	0.213	0.243	0.188	0.165	0.257	0.222	0.175	0.219	0.204	0.174	0.217	0.157	0.208	0.163	0.273	0.150

**Table 4:** Effect of inoculum concentrations, strains and storage period on ascorbic acid of mango wine samples

Storage Period (Days)	Ascorbic Acid(mg/100ml)																	
	Inoculum Concentration (5%)						Inoculum Concentration (10%)						Inoculum Concentration (15%)					
	IARI Strain			Native Strain			IARI Strain			Native Strain			IARI Strain			Native Strain		
	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G
0	34.50	24.00	28.35	34.50	24.00	28.35	34.45	24.00	28.35	34.44	24.00	28.35	34.50	24.00	28.35	34.50	24.00	28.35
15	31.40	21.25	25.40	31.42	21.24	25.40	31.42	21.20	25.42	31.42	21.20	25.40	31.44	21.18	25.42	31.42	21.20	25.40
30	28.45	18.50	22.55	28.45	18.50	22.54	28.48	18.45	22.52	28.46	18.45	22.54	28.48	18.42	22.50	28.50	18.42	22.48
45	24.25	14.65	19.54	24.26	14.65	19.54	24.24	14.62	19.52	24.24	14.62	19.54	24.28	14.60	19.52	24.30	14.62	19.52
60	20.60	11.80	15.80	20.60	11.78	15.80	20.55	11.76	15.76	20.50	11.76	15.80	20.50	11.74	15.74	20.45	11.74	15.74
CD	0.118	0.187	0.170	0.136	0.155	0.170	0.125	0.116	0.148	0.138	0.116	0.170	0.125	0.094	0.119	0.100	0.789	0.179
SE(d)	0.052	0.083	0.075	0.060	0.069	0.075	0.055	0.051	0.065	0.061	0.051	0.075	0.055	0.042	0.053	0.044	0.350	0.079
SE(m)	0.037	0.059	0.053	0.043	0.049	0.053	0.039	0.036	0.046	0.043	0.036	0.053	0.039	0.029	0.037	0.031	0.247	0.056
CV	0.231	0.562	0.412	0.265	0.466	0.412	0.243	0.349	0.359	0.269	0.349	0.412	0.244	0.283	0.290	0.195	2.379	0.434



**Table 5:** Effect of inoculum concentrations, strains and storage period on total plate count (TPC) of mango wine samples

Storage Period (Days)	Total plate count (log 10cfu/ml)																	
	Inoculum Concentration (5%)						Inoculum Concentration (10%)						Inoculum Concentration (15%)					
	IARI Strain			Native Strain			IARI Strain			Native Strain			IARI Strain			Native Strain		
	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G	D	C	G
0	6.95	7.06	7.04	6.94	7.05	7.04	6.96	7.05	7.04	6.95	7.06	7.04	6.96	7.07	7.05	6.94	7.05	7.05
15	4.90	5.05	5.03	4.90	5.04	5.02	4.88	5.02	5.01	4.89	5.04	5.04	4.80	4.95	4.96	4.81	4.94	5.04
30	2.50	2.65	2.60	2.52	2.64	2.58	2.45	2.62	2.56	2.44	2.61	2.56	2.40	2.58	2.52	2.41	2.62	2.56
45	0.80	0.90	0.85	0.80	0.91	0.84	0.78	0.88	0.83	0.78	0.90	0.83	0.76	0.86	0.84	0.77	0.88	0.82
60	1.40	1.45	1.46	1.41	1.46	1.45	1.41	1.46	1.47	1.42	1.45	1.46	1.43	1.45	1.46	1.43	1.47	1.46
CD	0.029	0.036	0.040	0.043	0.049	0.040	0.044	0.053	0.039	0.063	0.054	0.050	0.067	0.063	0.047	0.049	0.051	0.049
SE(d)	0.013	0.016	0.018	0.019	0.022	0.018	0.019	0.023	0.017	0.028	0.024	0.022	0.030	0.028	0.021	0.022	0.023	0.022
SE(m)	0.009	0.011	0.013	0.013	0.015	0.012	0.014	0.017	0.012	0.020	0.017	0.016	0.021	0.020	0.015	0.015	0.016	0.015
CV	0.468	0.570	0.645	0.701	0.785	0.633	0.718	0.841	0.620	1.033	0.852	0.803	1.111	1.007	0.763	0.809	0.823	0.784

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