AACCI Approved Methods Technical Committee Report: Collaborative Study on a Method for Determining Firmness of Cooked Pulses (AACCI Method 56-36.01)

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ABSTRACT

A method based on compression through a Kramer shear cell was developed for determining the firmness of cooked pulses. Ten laboratories analyzed twenty-six blind duplicates of thirteen different samples in a collaborative study to evaluate the repeatability and reproducibility of the method. Statistical analysis of the collaborative data indicated that within-laboratory repeatability standard deviation (s_r) was in the range of 0.53 to 1.43, and among-laboratory reproducibility standard deviation (s_R) varied from 0.74 to 1.94. The within-laboratory relative standard deviation (RSD_r) of samples ranged from 2.45 to 7.24%, and the among-laboratory relative standard deviation (RSD_R) ranged from 4.23 to 8.80%.

Cooking quality is an important quality characteristic for pulses (peas [*Pisum sativum*], lentils [*Lens culinaris*], chickpeas [*Cicer arietinum*], and beans [*Phaseolus vulgaris*]) because they are commonly consumed after cooking in various types of foods. Cooking is required to ensure acceptable sensory quality. Texture/firmness is one of the most important quality factors influencing consumer acceptance of cooked pulses. Thus, evaluation of the texture/firmness of cooked pulses is critical to the determination of cooking quality. There are several methods available for measuring the texture/firmness of cooked pulses, both subjective and objective, but no universally accepted method exists. One subjective method for measuring firmness evaluates the tenderness of cooked pulses using a trained sensory panel (10). In another subjective method, firmness is measured by squeezing the cooked seeds between the thumb and finger (5,9). Objective or instrumental methods for determining the texture/firmness of cooked pulses include texture profile analysis using a texture analyzer, puncture tests that measure the force required to penetrate individual cooked seeds, and compression tests (4,8,12).

Compression tests encompass a wide variety of test methods and operating parameters. Researchers have measured the force required to compress a single layer of cooked pulses between two plates (3,17). More common compression testing methods include a shear compression cell equipped with an extrusion grid (6,7,14) and the Kramer shear cell, which consists of a number of blades that pass through the sample (2,11,15,18). Methods for compression testing depend on the equipment used and highly variable operating parameters, including the sample size, load cell size, crosshead speed, and extent to which the sample is compressed. The variability within these parameters makes it difficult to compare results. Therefore, there is a need to develop a standard method for measuring firmness of cooked pulses.

The objectives of this study were to develop a standard method for determining the firmness of cooked pulses and to determine the precision of the method. An initial collaborative trial for the method developed (19), which included a small number of participating laboratories, was presented to the AACC International (AACCI) Pulse and Grain Legume Technical Committee in 2010. The results were sufficiently favorable to allow recommendation of a full collaborative study.

Materials and Methods

A single bulk sample for each of 13 different pulse samples, including 3 yellow pea, 1 lentil, 3 Kabuli chickpea, 3 navy bean, and 3 pinto bean samples, was obtained from a local processor in Canada. Each sample was mixed thoroughly and then split into two bulk samples using a Boerner sample divider (Seedburo Equipment Company), resulting in blind duplicates. A portion from each sub-bulk sample was packaged into individual plastic bags and identified with a different random four digit number. Twenty-six blind duplicates of thirteen different samples were sent to ten participants for the determination of cooked pulse firmness.

The procedure for determining the firmness of cooked pulse samples and instructions for the collaborative study were provided to the participants. Participants were instructed to follow the procedure and instructions exactly. The cooking time for each sample was predetermined using an automated Mattson cooker (16) and supplied to each participant.

The firmness of cooked pulse samples was determined using a texture analyzer (TA-XT2, TA-XT Plus, or TA-HDi, Texture Technologies Corp.) with a load cell capacity of 25, 50, 100, or 250 kg. Briefly, a 40 g sample was soaked in 160 mL of distilled water at room temperature for 24 hr. After draining the water, the soaked sample was cooked in 1.0 L of distilled water in a 2 L metal beaker placed on a hot plate for its predetermined cooking time. The cooked sample was drained for 15 sec using a strainer, and then the strainer was placed in a plastic container holding 700 mL of distilled water (20 ± 2°C) for 30 sec. The cooked seeds were drained and transferred to another plastic container holding 700 mL of distilled water (20 ± 2°C) for an additional 90 sec to cool the cooked seeds to room temperature. The cooked seeds were drained and transferred to a 250 mL capacity container to keep the seeds at room temperature. Approximately 7.5 ± 0.5 g of cooked sample was loaded on a Kramer shear cell holder (TA-91M, Texture Technologies Corp.). The Kramer shear cell was attached to the load cell of the texture analyzer. System parameters were 1) 2.0 mm/sec arm speed prior to sample contact; 2)

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1.5 mm/sec arm speed during compression of the sample; and 3) 2.0 mm/sec arm speed during retraction. The maximum shear force measured was recorded (Fig. 1). The firmness of the cooked sample was defined as the maximum force required to shear the cooked sample and expressed as the maximum shear force per gram of cooked sample (N/g of cooked sample). Firmness values reported are an average of six determinations.

The results were analyzed, and outlying results were detected according to Nelsen and Wehling (13) and AOAC (1), using a 2.5% significance level. A Cochran test was used to identify suspect replicate results within a laboratory, and a Grubbs test was used to determine whether the average results for a laboratory deviated from the average results of all laboratories. Cycles of Cochran, single Grubbs, and pair Grubbs tests were used to identify outliers until no additional removal was necessary or no more than two of nine laboratories were flagged. A rank test was performed to determine whether one or more laboratories were consistently low or high with respect to the other laboratories, and ranks were tested for significance at $P < 0.05$ (13,20). Analysis of variance (ANOVA) for the main effects (sample and laboratory) and interaction (sample × laboratory) was also determined using the GLM procedure (version 9.2, SAS Institute).

Results and Discussion

A preliminary collaborative study was conducted in 2010 (19), and results indicated that a standard deviation of repeatability (s_r) in the range of 0.5 to 2.3 and a standard deviation of reproducibility (s_R) in the range of 1.6 to 4.2 could be achieved. Although these results were encouraging, the number of participants in the study was relatively small. The AACCI Pulse and Legume Technical Committee recommended to the AACCI Approved Methods Technical Committee that a full collaborative study with a greater number of participating laboratories be performed to confirm the results.

Table I displays the results from this full collaborative study. Examination of the data from this study using Cochran and Grubbs tests indicated that the results from lab 3 for the Kabuli chickpea 2 sample and from lab 6 for the lentil sample were outliers. The Cochran test was used to check for outliers in individual measurements, and the Grubbs test was used to check for

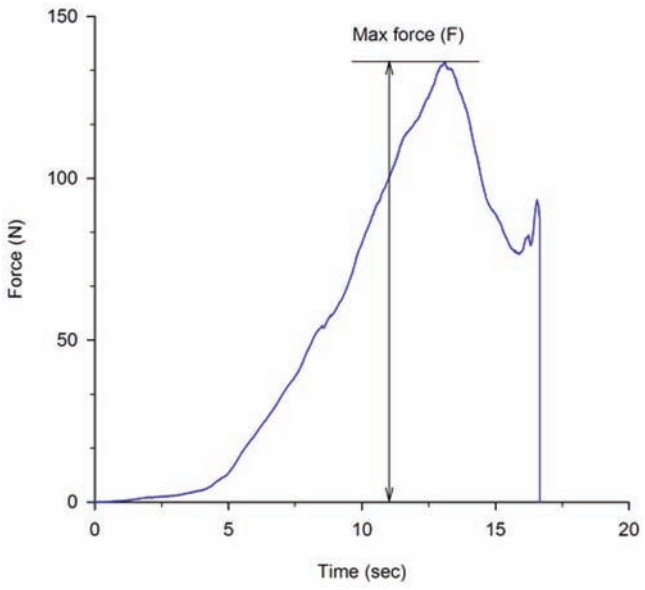

Fig. 1. Typical plot for determining firmness of cooked pulses.

Table I. Results of the full collaborative study on determination of firmness of cooked pulses (N/g of cooked sample)

		Collaborating Laboratory									
Sample	**Duplicate**	**1**	**2**	**3[a]**	**4**	**5[a]**	**6**	**7**	**8**	**9**	**10**
Chickpea 1	1	27.8	26.4	30.4	26.8	23.9	28.1	26.9	28.7	29.7	28.7
	2	27.2	24.5	33.7	26.2	24.3	26.5	27.1	26.2	29.5	26.7
Chickpea 2	1	25.8	24.6	28.5[b]	25.2	21.8	25.4	24.1	25.6	27.5	24.2
	2	24.2	23.9	32.8[b]	25.6	22.5	27.0	24.9	25.5	26.7	25.5
Chickpea 3	1	21.1	21.1	26.3	22.9	21.1	25.5	21.7	23.9	23.5	22.5
	2	21.2	22.5	27.6	21.9	20.8	23.7	21.4	22.7	25.9	21.2
Lentil	1	14.2	16.6	14.8	14.7	16.7	18.3[c]	14.2	15.7	15.8	14.4
	2	15.8	14.8	14.6	15.0	15.6	19.1[c]	14.3	15.3	15.5	14.6
Navy bean 1	1	18.0	18.8	24.6	18.1	16.4	20.4	19.7	22.0	23.0	19.5
	2	21.1	20.1	24.8	18.4	19.2	17.7	19.4	20.1	19.9	20.4
Navy bean 2	1	22.4	19.9	26.2	22.6	17.7	19.6	22.6	23.0	26.4	21.3
	2	23.2	20.8	26.7	20.3	20.0	20.9	21.3	21.1	25.6	21.4
Navy bean 3	1	22.5	21.9	25.4	22.2	19.5	22.1	21.7	23.2	23.6	22.8
	2	21.0	23.1	26.9	20.7	18.2	22.6	22.5	24.0	24.8	23.3
Pea 1	1	21.2	18.0	19.3	19.1	18.2	21.8	17.5	21.3	19.5	18.1
	2	19.5	18.5	19.3	18.0	17.4	21.4	18.8	20.4	20.3	17.9
Pea 2	1	20.1	20.5	22.4	20.3	21.5	22.2	20.2	22.0	21.0	20.0
	2	22.5	21.3	23.7	19.7	20.7	24.5	19.4	24.9	22.8	19.8
Pea 3	1	18.9	20.6	23.0	19.2	19.2	21.9	18.6	19.3	22.7	18.9
	2	17.2	20.7	22.0	20.0	19.2	19.9	19.3	20.9	21.3	19.3
Pinto bean 1	1	26.2	24.5	27.9	22.8	23.0	25.5	25.2	27.5	26.1	24.4
	2	24.0	25.2	28.7	24.9	22.4	25.3	24.5	27.0	25.5	23.5
Pinto bean 2	1	20.8	20.9	26.6	21.0	19.1	23.2	21.6	22.7	24.5	21.5
	2	21.8	20.4	24.8	21.5	19.4	21.6	21.2	22.2	24.9	21.6
Pinto bean 3	1	24.5	23.1	26.9	21.2	18.2	23.3	21.7	21.9	24.6	21.7
	2	21.4	20.0	28.3	20.5	18.8	20.6	21.1	22.3	24.3	22.6

[a] Data excluded from the analysis.
[b] Outlier detected by Cochran test.
[c] Outlier detected by single Grubbs test.

laboratory outliers (13). A method that produces outliers at a greater than two to nine ratio is considered unstable.

A rank test was carried out on data collected from the collaborative study by assigning ranks to the collaborators based on the firmness values for each sample from largest to smallest. Rank 1 was given to the highest firmness value for any sample studied, rank 2 to the next highest, and so on. Each collaborator's ranks over the number of samples studied were then added up. The rank sum was tested for significance at $P < 0.05$. For 10 labs and 13 samples, no rank sum should be ($P < 0.05$) <43 or >100 (20). The sum of ranks from this collaborative study is presented in Figure 2. Labs 3 and 5 fell outside the limits of 43 and 100, corresponding to a 95% confidence interval. Results indicated that lab 3 had consistently high firmness values. It is well known that growing and storage conditions render pulses susceptible to a hardening phenomenon known as the hard-to-cook (HTC) defect. Pulses with the HTC defect remain entirely or partially unswollen or unhydrated after cooking. A detailed procedure, including a PowerPoint presentation, for determining firmness of cooked samples and instructions for the collaborative study were provided to the participants. According to the test procedure HTC seeds need to be removed after cooking. Investigation found that lab 3 had difficulty differentiating HTC seeds from cooked pulse samples during testing due to inexperience in carrying out the test. Preliminary results from the study coordinator's lab showed that samples with HTC seeds resulted in higher firmness values compared with those with HTC seeds removed. Therefore, inexperience in identifying partially unswollen or unhydrated seeds in cooked samples would result in higher firmness values. Another source of errors for lab 3 that resulted in consistently higher values could be Kramer shear blades slightly touching the sample holder cell during measurements. Lab 5, on the other hand, showed the highest sum of ranks, indicating that it reported consistently low firmness values (Fig. 2). Lab 5 commented that it observed unhydrated seeds in some cooked samples and that the cooked samples had to be picked through to select for testing. As a result, both partially unhydrated seeds and non-HTC seeds in cooked samples might be removed prior to testing. This would result in consistently low firmness values measured in cooked pulse samples. Based on these results, data from labs 3 and 5 were considered invalid or outliers and were excluded from the analysis. The performance parameter statistics obtained after removal of outliers are shown in Table II.

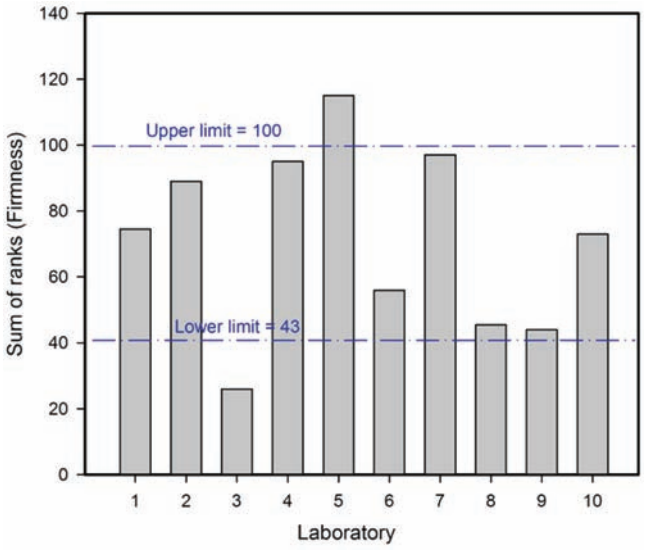

Fig. 2. Sum of ranks for firmness values for collaborating laboratories.

The repeatability standard deviation within laboratories (s_r) for the pulse samples ranged from 0.54 to 1.43, with an average of 0.93, and the reproducibility standard deviation among laboratories (s_R) varied from 0.74 to 1.94, with an average of 1.38 (Table II). The within-laboratory relative standard deviation (RSD_r) ranged from 2.45 to 7.24%, and the among-laboratory relative standard deviation (RSD_R) ranged from 4.23 to 8.80%. The repeatability value (r) ranged from 1.51 to 4.01. Repeatability is the internal precision of a method (13): two single results obtained within a laboratory under repeatable conditions (same technician using the same instruments in the same laboratory at the same time) should not differ by more than r. The reproducibility value (R) ranged from 2.07 to 5.43. Reproducibility is the external precision of a method: two single results obtained by two different laboratories under reproducible conditions (different technicians using different instruments in different laboratories at different times) should not differ by more than R. Given the variables among laboratories (differing equipment and slightly different applications of the method by technicians), reproducibility incorporates a many of the variables that are likely to be encountered in common use of a method. The reproducibility value obtained from the collaborative study is expected to be greater than the repeatability value.

As an additional measure of the quality of the method, the level of significance of the interaction term between sample and laboratory was obtained by ANOVA. Results showed there was

Table II. Statistical data for the full collaborative study on determination of firmness of cooked pulses (outliers removed)[a]

Sample	No. of Labs	Mean	s_r	s_R	RSD_r	RSD_R	r	R
Chickpea 1	8	27.3	1.04	1.49	3.80	5.07	2.90	3.88
Chickpea 2	8	25.4	0.74	1.07	2.92	4.23	2.07	3.00
Chickpea 3	8	22.7	0.97	1.54	4.30	6.80	2.73	4.31
Lentil	7	15.1	0.67	0.74	4.41	4.92	1.86	2.07
Navy bean 1	8	19.8	1.43	1.45	7.24	7.32	4.01	4.06
Navy bean 2	8	22.0	0.95	1.94	4.30	8.80	2.65	5.43
Navy bean 3	8	22.6	0.76	1.08	3.34	4.76	2.12	3.02
Pea 1	8	19.5	0.69	1.48	3.56	7.60	1.94	4.12
Pea 2	8	21.3	1.23	1.71	5.79	8.04	3.46	4.80
Pea 3	8	19.9	0.89	1.41	4.47	7.06	2.50	3.94
Pinto bean 1	8	25.1	0.85	1.24	3.40	4.93	2.39	3.47
Pinto bean 2	8	22.0	0.54	1.31	2.45	5.98	1.51	3.68
Pinto bean 3	8	22.2	1.33	1.45	6.01	6.56	3.73	4.07

[a] s_r = repeatability standard deviation; s_R = reproducibility standard deviation; RSD_r = repeatability relative standard deviation; RSD_R = reproducibility relative standard deviation; r = repeatability value ($2.8 \times s_r$); and R = reproducibility value ($2.8 \times s_R$).

no significant interaction between sample and laboratory for firmness values measured (Table III). This indicated that the method was consistent across all materials for all laboratories.

To qualitatively determine how well the method separated various pulse samples, Duncan's multiple range test was run (Table IV). Results indicated that the method produced good separation among samples differing in firmness. No correlation between the precision values (s_r and s_R) and the mean firmness values was observed (Fig. 3).

Conclusions

In conclusion, the method developed for determining the firmness of cooked pulses had an s_r value between 0.54 and 1.43 and an s_R value between 0.74 and 1.94. The results of the collaborative study demonstrated that cooked samples with different firmness values could be successfully differentiated from each other. The study also revealed that care must be taken when picking up entirely or partially unswollen seeds in cooked samples. Experience and practice in running the test are recommended to obtain consistent results. Based on the results of the collaborative study, we recommend that this method be granted Final Approval status by the AACCI Approved Methods Technical Committee.

Table III. Analysis of variance of firmness values from the collaborative study (outliers removed)

Source	Degree of Freedom	Mean Square	*F* Value	*Pr*
Sample	12	144.5	154.1	<0.0001
Lab	7	24.1	25.7	<0.0001
Sample × lab	83	1.24	1.32	0.0913

Table IV. Duncan's multiple range test for separation of pulse samples in the collaborative study (outliers removed)

Sample	Duncan's Multiple Range Test (mean)[a]
Chickpea 1	27.3 a
Chickpea 2	25.4 b
Pinto bean 1	25.1 b
Chickpea 3	22.7 c
Navy bean 3	22.6 c
Pinto bean 3	22.2 c
Navy bean 2	22.0 cd
Pinto bean 2	21.9 cd
Pea 2	21.3 d
Pea 3	19.9 e
Navy bean 1	19.8 e
Pea 1	19.5 e
Lentil	15.1 f

[a] Means followed by the same letter are not significantly different ($P < 0.05$).

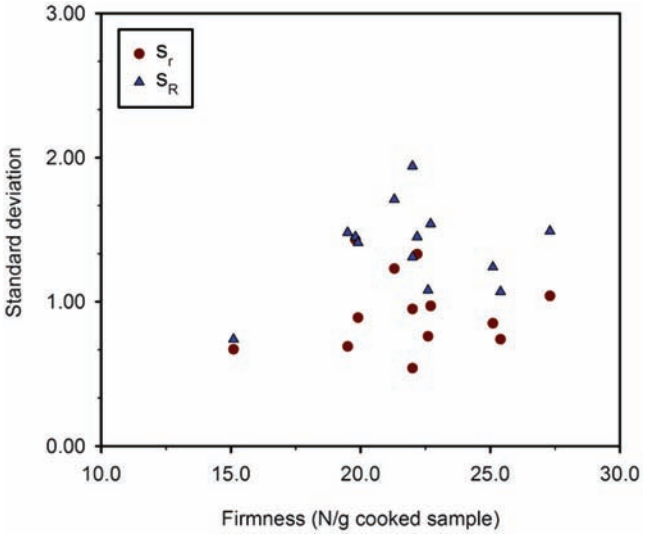

Fig. 3. Relationship between precision values (repeatability standard deviation [s_r] and reproducibility standard deviation [s_R]) and mean firmness of cooked pulses.

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References

1. AOAC. Guidelines for collaborative study procedures to validate characteristics of a method of analysis. J. Assoc. Off. Anal. Chem. 72:694, 1989.
2. Bhatty, R. S. Cooking quality of lentils: The role of structure and composition of cell walls. J. Agric. Food Chem. 38:376, 1990.
3. Buckle, K. A., and Sambudi, H. Effect of soaking and boiling treatments on the quality of winged bean seed. J. Sci. Food Agric. 53:379, 1990.
4. Chenoll, C., Betoret, N., and Fito, P. Analysis of chickpea rehydration. Part 1. Physicochemical and texture analysis. J. Food Eng. 95:352, 2009.
5. Coskuner, Y., and Karabab, E. Effect of location and soaking treatments on the cooking quality of some chickpea breeding lines. Int. J. Food Sci. Technol. 38:751, 2003.
6. Garcia-Vela, L. A., Del-Valle, J. M., and Stanley, D. W. Hard-to-cook defect in black beans: The effect of soaking in various aqueous salt solutions. Can. Inst. Food Sci. Technol. J. 24:60, 1991.
7. Garcia-Vela, L. A., and Stanley, D. W. Protein denaturation and starch gelatinization in hard-to-cook beans. J. Food Sci. 54:1284, 1989.
8. Iliadis, C. Influence of genotype and soil type on cooking time in lentil (*Lens culinaris*). Int. J. Food Sci. Technol. 38:89, 2003.
9. Kaur, S., Singh, N., Sodhi, N. S., and Rana, J. C. Diversity in properties of seed and flour of kidney bean germplasm. Food Chem. 117:282, 2009.
10. Mkanda, A. V., Minnaar, A., and de Kock, H. L. Relating consumer preferences to sensory and physicochemical properties of dry beans (*Phaseolus vulgaris*). J. Sci. Food Agric. 87:2868, 2007.
11. Moharram, Y. G., Abou-Samaha, A. R., and El-Mahady, A. R. Effect of cooking methods on the quality of lentils. Z. Lebensm.-Unters. -Forsch. 182:307, 1986.
12. Mwangwela, A. M., Waniska, R. D., McDonough, C., and Minnaar, A. Cowpea cooking characteristics as affected by micronization temperature: A study of the physicochemical and functional properties of starch. J. Sci. Food Agric. 87:399, 2007.
13. Nelsen, T. C., and Wehling, P. Collaborative studies for quantitative chemical analytical methods. Cereal Foods World 53:285, 2008.
14. Sefa-Dedeh, S., Stanley, D. W., and Voisey, P. W. Effect of soaking time and cooking conditions on texture and microstructure of cowpeas (*Vigna unguiculata*). J. Food Sci. 43:1832, 1978.

15. Tang, J., Sokhansanj, S., and Sosulski, F. W. Lentils quality—Effects of artificial drying and six-month storage. Can. Inst. Food. Sci. Technol. J. 24:283, 1991.
16. Wang, N., and Daun, J. K. Determination of cooking times of pulses using an automated Mattson cooker apparatus. J. Sci. Food Agric. 85:1631, 2005.
17. Wang, N., Daun, J. K., and Malcolmson, L. J. Relationship between physicochemical and cooking properties and effects of cooking on antinutrients of yellow field peas (*Pisum sativum*). J. Sci. Food Agric. 83:1228, 2003.
18. Wang, N., Hatcher, D. W., Warkentin, T. D., and Toews, R. Effect of cultivar and environment on physico-chemical and cooking characteristics of field pea (*Pisum sativum*). J. Food Chem. 118:109, 2010.
19. Wang, N., Malcolmson, L. J., Panozzo, J. F., and Arganosa, G. C. Preliminary collaborative study of a method for determining firmness of cooked pulses. Canadian Grain Commission, Winnipeg, Canada, 2010.
20. Wernimont, G. T. *Use of Statistics to Develop and Evaluate Analytical Methods*. W. Spendley, ed. Association of Official Analytical Chemists, Gaithersburg, MD, 1985.

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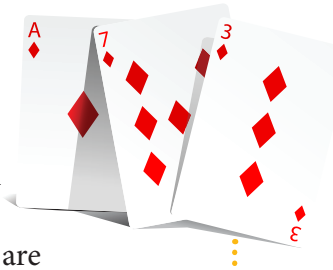

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