



## **Resistant Starch Content of Traditional Indian Legume Preparations**

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### **Abstract**

Foods rich in resistant starch (RS) can play an important role in the management of chronic diseases. Legumes contain higher amount of RS than cereals, roots and tubers. Therefore the current study was undertaken to estimate RS content of some commonly consumed Indian legume preparations. 26 cooked legume based products including 6 readymade traditional snacks were tested for their RS content using Megazyme Assay Kit for Resistant Starch. RS content was estimated for 100 g cooked food and for the cooked portion obtained by using 30 g of major ingredient. One way ANOVA with post hoc Tukey's test was used to compare the mean RS content in one portion of different categories of legume preparations. Level of significance was set at  $p < 0.05$ . RS content of four types of plain pressure cooked *dhal*, common to Indian cooking, was in the range of 1.21 g % to 2.16 g % whereas *aamti*, a thinner preparation of *dhal* contained RS in the range of 0.42 g % to 0.69 g %. In both types of *dhal* preparations, red gram *dhal* showed highest RS content followed by bengal gram *dhal*. Usal made using bengal gram (*kabuli*) contained 2.38 g % RS followed by 1.42 g % RS in bengal gram (brown) usal. RS in bengal gram flour based traditional recipes like *zunka*, *dhirda* and *pithle* was 1.23 g %, 1.48 g % and 0.09 g % respectively. When mean RS content of one portion of different categories of legume preparations was compared, there was no significant difference ( $p \geq 0.05$ ) found among any of the categories. The results of the study generated useful data with respect to RS content in 100 g as well as RS in one portion of legume preparations and identified preparations with red gram *dhal*, bengal gram (*kabuli* and brown variety) and lentil as relatively higher in RS content.



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## Introduction

Resistant starch (RS) is the sum of starch and products of starch degradation that are not absorbed in the small intestine of healthy individuals<sup>1,2</sup>. It is fermented in the large intestine of humans, with the production of short-chain fatty acids<sup>3</sup>. Different types of RS ranging from RS1 to RS5 have been discussed in literature<sup>1,2,4,5,6,7</sup>. The potential health benefits of consumption of RS have been highlighted in many studies. Its role in improving bowel health<sup>1,8,9,10</sup>, decreasing food and energy intake<sup>11,12</sup>, improving blood lipids<sup>13,14,15</sup> and improving glucose and insulin responses<sup>16,17,18,19,20</sup> in animals and humans have been demonstrated by many researchers.

Foods rich in RS, thus, can play an important role in the management of chronic diseases. Studies indicate that legumes can contribute significantly to the RS content of the diet as they contain higher amount of RS than cereals, roots and tubers<sup>21</sup>. RS content in the legume products varies depending on the variety, type of processing and thermal treatment given<sup>22</sup>. In Indian diet, variety of legumes is used as whole grains, as decorticated split legumes (*dhals*) or in the form of legume flour, in different preparations and there is a need to generate the data base with respect to their RS content. The data so generated, should have practical applicability so that these RS values can be used while analysing diet data in large food consumption studies to estimate RS intake by the study population or for the purpose of planning RS rich diets. Therefore, the current study was undertaken to estimate RS content of some commonly consumed Indian legume preparations and to identify the preparation with relatively higher RS content.

## Materials and Methods

### Sample

Preparations made from four types of *dhal*, seven types of whole grain legumes as well as bengal gram flour preparations, which are consumed in a typical Indian diet, were included in the study. In all, 26 cooked products including 6 readymade traditional snacks were tested for their RS content. Samples of each of the *dhal*, legumes, bengal gram flour as well as readymade snacks were procured from three market places located in Mumbai, which were then mixed in equal proportions to obtain a homogenous

mixture. This was then used in the preparation of *dhal* and legume products which were prepared using the regular cooking methods commonly used in Indian cooking.

### Plain Pressure Cooked *Dhal*

Decorticated split legumes were cooked in the pressure cooker after adding water in 1:2 (w/v) ratios. Salt was added to the cooked *dhal* and it was mashed to obtain a plain *dhal* with thick consistency. In case of bengal gram *dhal* with spinach, pressure cooked bengal gram *dhal* was added to spinach after sautéing spinach using oil and Indian spices.

### *Aamti*

The traditional preparation of *aamti* was made using *dhal* that was first pressure cooked adding water in 1:2 (w/v) ratios. The cooked *dhal* was then seasoned with oil and Indian spices, and the desired consistency was obtained by adding extra water. Onions and/or tomato were used as per the requirement of the recipe.

### *Usal*

Seven types of whole legumes, either sprouted or without sprouting, were used to prepare *usal* which is a traditional Indian preparation. Two varieties of green gram *usal* were prepared, one using sprouted green gram and the other using green gram without soaking and sprouting. Moth beans and field beans were soaked and sprouted before preparing *usal*, where as the rest of the legumes were just soaked and used in *usal*. In general the legumes were soaked for 8 to 10 hours and used or were allowed to sprout for 24 hrs after soaking. After pressure cooking, the processed legumes were seasoned using oil and Indian spices. Onions and/or tomato were added as per the requirement of the recipe.

*Zunka*, *dhirda* and *pithle* were prepared using bengal gram flour, following the traditional method of cooking these products and the rest of the products included in the study were purchased readymade from the market.

The amount of raw ingredients used and the final cooked weight of the preparation were recorded carefully.

**Determination of Resistant Starch**

Resistant starch content was determined by using Megazyme Assay Kit for Resistant starch. The procedure was based on AOAC method 2002.02<sup>23</sup>. Assay kit was purchased from Megazyme International Limited, Ireland. Cooked samples were ground into a paste after cooling them to room temperature and were then subjected to the procedure of estimating RS.

Food samples, weighing approximately 500 mg, were incubated in a shaking water bath with pancreatic  $\alpha$ -amylase and amyloglucosidase (AMG) for 16 hrs at 37 °C. Equal volume of ethanol was then added and the RS was recovered as a pellet on centrifugation. This was then washed twice by suspension in ethanol (50 %v/v), followed by centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2M KOH by vigorously stirring in an ice water bath over a magnetic stirrer. This solution was neutralised with acetate buffer and the starch was quantitatively hydrolysed to glucose with AMG. D-Glucose was measured with glucose oxidase / peroxidase reagent (GOPOD). RS (g % in the sample 'as eaten') was calculated.

RS (g) per 100 g cooked food = glucose concentration in food sample (as obtained from standard curve)

$\times 0.9 \times \text{volume correction} \times 1/1000 \times 100/\text{sample weight}$

**Statistical Analysis**

The RS content in grams per 100 g cooked food 'as eaten' was expressed as mean  $\pm$  SD. 30 g of most of the legumes would be equivalent to approximately one 100 kcal exchange, which, in the current study was considered as one portion. Therefore, the RS content in the portion of food prepared using 30 g of major (raw) ingredient was also computed. One way ANOVA with post hoc Tukey's test was used to compare the mean RS content in one portion of different categories of legume preparations. Level of significance was set at  $p < 0.05$ .

**Results**

RS content of *dhal*, legume and bengal gram flour based preparations was estimated in the current study. Table 1 shows RS content of *dhal* preparations. This included plain, pressure cooked *dhal* with thick consistency and *dhal aamti* with relatively thinner consistency. The RS values are given as mean  $\pm$  SD. The table also gives the amount of *dhal* required to prepare 100 g cooked product. RS content in the table is expressed as RS g /100 g cooked food 'as eaten'.

**Table 1: Resistant starch content of *dhal* preparations**

Food product and description	Amount of raw <i>dhal</i> per 100 g cooked product(g)	RS in 100 g cooked product (g)
<b>Plain pressure cooked <i>dhal</i></b>		
Red gram <i>dhal</i> - thick consistency	33	2.16 $\pm$ 0.06
Green gram <i>dhal</i> - thick consistency	33	1.43 $\pm$ 0.02
Bengal gram <i>dhal</i> - cooked but not mashed	43	1.57 $\pm$ 0.02
Bengal gram <i>dhal</i> with spinach- <i>dhal</i> not mashed	21	0.79 $\pm$ 0.02
Black gram <i>dhal</i> – thick consistency	38	1.21 $\pm$ 0.03
<b><i>Aamti</i></b>		
Red gram <i>dhal aamti</i> - medium thick	20	0.69 $\pm$ 0.02
Green gram <i>dhal aamti</i> - medium thick	16	0.42 $\pm$ 0.02
Bengal gram <i>dhal aamti</i> – medium thick	19	0.63 $\pm$ 0.03
Black gram <i>dhal aamti</i> - medium thick	17	0.50 $\pm$ 0.03

RS value presented as Mean  $\pm$  SD

RS in plain pressure cooked dhal varied from  $1.21 \pm 0.03$  g % in black gram *dhal* to  $2.16 \pm 0.06$  g % in red gram *dhal* with green gram and bengal gram *dhal* having intermediate values of  $1.43 \pm 0.02$  g % and  $1.57 \pm 0.02$  g % respectively. Bengal gram dhal was also combined with spinach and it contained  $0.79 \pm 0.02$  g % RS. The same *dhals* were also used to prepare *aamti* and the RS in *aamti* ranged from  $0.42 \pm 0.02$  g % in green gram *dhal aamti* to  $0.69 \pm 0.02$  g % in red gram *dhal aamti* with bengal

gram *dhal* and black gram *dhal aamti* having  $0.63 \pm 0.03$  g % and  $0.50 \pm 0.03$  g % RS respectively.

Table 2 shows RS content of legume preparations. This includes usual prepared using whole legumes and some traditional preparations made using bengal gram flour. The RS values are given as mean $\pm$ SD. The table also gives the amount of legumes or bengal gram flour required to prepare 100 g cooked product. RS content in the table is expressed as RS g / 100 g cooked food 'as eaten'.

**Table 2: Resistant starch content of legume preparations**

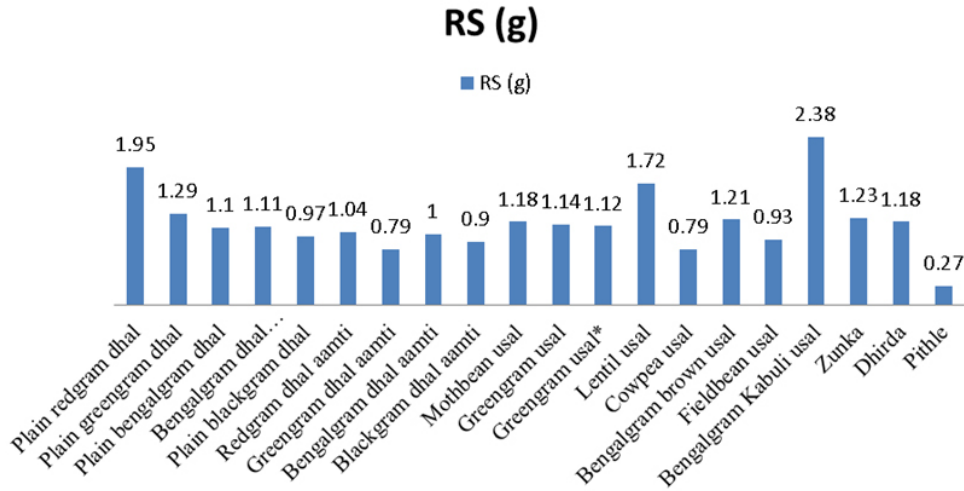
Food product and description	Amount of raw ingredient per 100g cooked product (g)	RS in 100g cooked product(g)
<b>Usal made with whole legumes</b>		
Moth bean- sprouted, pressure cooked, sautéed	24	$0.94 \pm 0.00$
Green gram- sprouted, pressure cooked, sautéed	23	$0.89 \pm 0.04$
Green gram- pressure cooked, sautéed	19	$0.70 \pm 0.02$
Lentil- soaked, pressure cooked, sautéed	21	$1.23 \pm 0.03$
Cowpea – soaked, pressure cooked, sautéed	31	$0.82 \pm 0.06$
Bengal gram (brown) - soaked, pressure cooked, sautéed	35	$1.42 \pm 0.03$
Field beans- sprouted, pressure cooked, sautéed	24	$0.74 \pm 0.02$
Bengal gram (kabuli)- soaked, pressure cooked, sautéed	30	$2.38 \pm 0.3$
<b>Ethnic food made using Bengal gram flour</b>		
<i>Zunka</i> - cooked with moist heat	30	$1.23 \pm 0.03$
<i>Dhirda</i> - batter shallow fried	38	$1.48 \pm 0.04$
<i>Pithle</i> - cooked with moist heat (medium thick)	10	$0.09 \pm 0.01$

RS value presented as Mean  $\pm$  SD

With an exception of bengal gram (*kabuli*) *usal* which contained  $2.38 \pm 0.3$  g % RS, the RS in the rest of the *usal* varieties was found to be in the range of  $0.70 \pm 0.02$  g % in green gram *usal* (not germinated) to  $1.42 \pm 0.03$  g % in bengal gram (brown) *usal*. As seen in the table, bengal gram (*kabuli* and brown) *usal* and lentil *usal* showed higher RS values as compared to *usal* made from other legumes. RS content of *zunka*, *dhirda* and *pithle* was  $1.23 \pm 0.03$  g %,  $1.48 \pm 0.04$  g % and  $0.09 \pm 0.01$  g % respectively.

As seen in Table 1 and Table 2, the quantity of *dhal* / legume (major ingredient) required in the raw form to obtain 100 g cooked product varied depending on the type of food preparation. This poses difficulty in comparing different legumes for their RS content. Therefore, RS content in portions of cooked food prepared from same quantity of raw dhal or legume was computed.

Fig 1 shows the RS in a portion of cooked food prepared using 30 g of raw *dhal* / legumes. These RS values were derived from the values indicated in Table1 and Table 2.

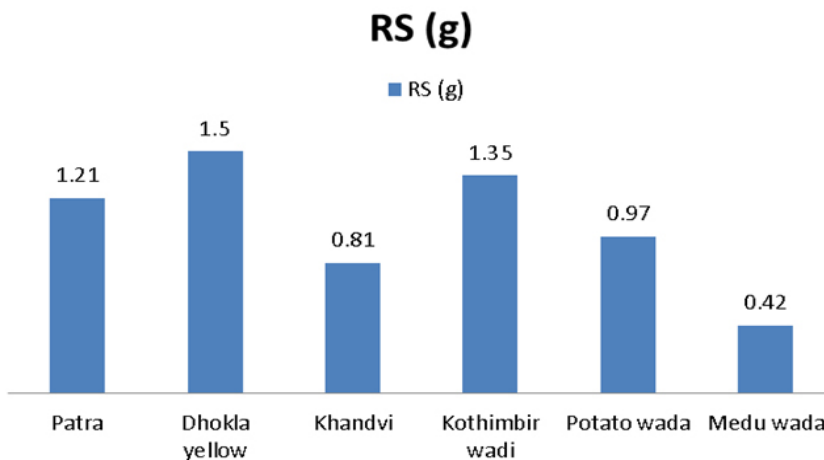


**Fig. 1: Resistant starch in cooked portion of *dhal*/ legume preparations prepared using 30g of major (raw) ingredient. \*Green gram *usal* without sprouting**

As seen in Figure 1, RS in one portion of different types of plain pressure cooked *dhal* ranged from 0.97 g in black gram *dhal* to 1.95 g in red gram *dhal*, with the values of 1.10 g and 1.11 g for bengal gram *dhal* and for vegetable prepared using 'bengal gram *dhal* and spinach' combination, respectively. RS in one portion of different aamti was between 0.79 g in green gram *dhal* aamti to 1.04 g in red gram *dhal* aamti whereas RS content of one portion of *usal* varied from 0.79 g in cowpea *usal* to 2.38 g in bengal gram (*kabuli*) *usal*. The two types of green gram *usal* showed similar RS content per portion, the values

being 1.14 g and 1.12 g RS for 'sprouted' and for 'not sprouted' *usal* respectively. The RS values for one portion of *zunka* and *dhirda*, were very close, 1.23 g and 1.18 g respectively, which were also close to RS in one portion of plain pressure cooked bengal gram *dhal* as well as *usal* (brown).

Fig 2 gives the RS content of 100 g (which was considered as one portion in this study) of some traditional snacks that were bought from the market.



**Fig. 2: Resistant starch in 100g readymade snacks**

Except for *medu vada* and potato *vada* the rest of the snacks contained bengal gram flour as the major ingredient and they were steamed products. The RS content in these products varied from 0.81 g % in *khandvi* to 1.50 g % in yellow *dhokla*. Potato *vada* with two RS sources (potato and bengal gram flour) also contained RS in the similar range (0.97 g %) whereas *medu vada* contained 0.42 g % RS.

**Table 3: Comparison of mean RS content in one portion of different categories of legume preparations**

Category(preparation type)	RS (g) Mean $\pm$ SD
Plain pressure cooked <i>dhal</i> #	1.284 $\pm$ 0.39
<i>Aamti</i> #	0.933 $\pm$ 0.11
<i>Usal</i> #	1.309 $\pm$ 0.51
Bengal gram flour products#	1.205 $\pm$ 0.04
Readymade Bengal gram based snacks*	1.168 $\pm$ 0.28

# Mean RS in cooked portion made using 30g raw *dhal*/legume; \*mean RS in 100g cooked snacks

Table 3 shows different categories of legume preparations. One way ANOVA with post hoc Tukey's test was used to compare the mean RS content of these categories of legume preparations. Except for the category of readymade snacks, for all other categories, the mean RS value indicated in the table was computed by considering the RS in one portion of each of the foods in the category, (values indicated in Figure 1). In case of readymade snacks mean RS was computed considering the RS in 100 g snacks (values indicated in Figure 2). *Pithle* and *Medu vada* were excluded from this comparison as *pithle* showed exceptionally low RS content and *medu vada* did not fit in any of the categories.

### Discussion

Traditional Indian diet includes variety of legumes in different forms. Plain pressure cooked red gram *dhal* (*Sadha varan*) with cooked rice is an integral part of traditional Maharashtrian meal. There are many vegetable preparations in which cooked *dhal* are added to enhance the palatability. Though data on RS content of some legumes is available in literature, there are not many studies that have estimated RS in

*dhal* preparations. RS content of pressure cooked red gram *dhal* and green gram *dhal* reported in one study was 8.4 $\pm$ 2.36 g and 8.6 $\pm$ 0.06 g / 100g respectively<sup>22</sup>. In the current study, among plain pressure cooked *dhal*, red gram *dhal* showed highest RS content (2.16  $\pm$  0.06 g %) followed by bengal gram *dhal*. But RS in the vegetable preparation 'bengal gram *dhal* with spinach' was found to be 0.79 $\pm$ 0.02 g% (Table1) which was almost half of the RS content of plain pressure cooked bengal gram *dhal*. This was expected, as the amount of *dhal* in 100 g vegetable preparation was approximately half the amount that was required to make 100 g plain pressure cooked *dhal* (Table 1).

The observed range of RS for *aamti* was much lower than the range observed for plain pressure cooked *dhal* (Table 1), but similar to plain pressure cooked *dhal*, among the four types of *aamti*, red gram *dhal aamti* showed highest RS followed by bengal gram *dhal aamti*. In order to achieve the desired, thinner consistency of *aamti*, extra water was added in *aamti* which resulted in lesser amount of *dhal* in 100 g *aamti* as compared to the amount in plain cooked *dhal*. Thus the concentration of starch in *aamti* was less than starch in plain cooked *dhal*. Literature suggests that concentration of starch in a preparation can be an important factor affecting RS 3 formation, higher starch concentration leading to more RS formation<sup>3,24</sup>.

With respect to legume preparations, RS values reported for cooked beans in the database by Murphy *et al.*,<sup>25</sup> were in the similar range as observed for *usal* in the current study with cooked/canned chickpeas in the database showing 2.6 g % RS, the value close to 2.38 $\pm$  0.3 g % RS in bengal gram (*kabuli*) *usal* (Table 2) in the current study. Chen *et al.*,<sup>26</sup> reported RS content of boiled soyabean, common cowpea and garden pea, consumed in Chinese diet, to be 1.27 g, 3.72 g and 3.84 g respectively in 100 g esculent. In contrast, much higher values have been reported for commonly consumed cooked legume products in Swedish diet<sup>27</sup>.

Among all types of *usal* in the current study, bengal gram (*kabuli* and brown) *usal* and lentil *usal* showed higher RS content (Table 2). A study by Yadav *et al.*,<sup>21</sup> on Indian legumes also reported higher RS in lentil and bengal gram as compared to RS in other

legumes used in their study. Thus bengal gram and lentil may be identified as legumes with relatively higher RS content. Germination appears to reduce RS content of legumes to various extent<sup>22, 28</sup>. Unlike green gram and moth beans which are generally germinated before preparing *usal*, bengal gram and lentil, most often, are used without germination, which could be one of the reasons for the higher RS values observed for these *usal*.

RS content of bengal gram flour based preparations, with the exception of *pithle* was also found to be similar to bengal gram *usal*. Very low RS content of *pithle* could be attributed to the high amount of water added in the recipe to achieve the desired semi-solid consistency which led to dilution of starch in the recipe.

When RS in one portion of various *dhal* and legume preparations was computed (Figure 1), pressure cooked red gram *dhal* showed highest (1.95 g) RS content. Interestingly, RS in the portion of bengal gram *dhal* and in the portion of vegetable with 'bengal gram *dhal* and spinach' combination, prepared using 30 g bengal gram *dhal*, was similar (1.10 g and 1.11 g respectively) suggesting that RS content of a food used in different recipes may be similar if the amount used and the heat treatment given is the same.

RS in one portion of *aamti* prepared using different types of *dhal* was between 0.79 g to 1.04 g, the observed range being lower than the range for one portion of plain pressure cooked *dhal* (Figure 1). In spite of using same quantity of raw *dhal* (30 g) there was a difference in the RS range of these two types of preparations suggesting that there could be some other factors, other than the amount of *dhal* used, that may influence the RS content of the product. *Aamti* is much thinner than plain cooked *dhal* in which starch is not densely packed. Therefore it may be easier for the enzymes to act on starch and cause its hydrolysis, reducing the RS content. In contrast, densely packed food like pasta is considered to be a source of RS<sup>13</sup> suggesting that densely packed foods may contain higher RS.

Though there was a difference in the RS content (per 100 g) of the two types of green gram *usal* (Table 2), both showed similar RS content in the

portion that was prepared using 30 g whole (raw) green gram (Figure 1), once again suggesting that the amount of starchy ingredient in a preparation would be one of the major factors affecting RS content of the recipe.

Snacks prepared using bengal gram flour are an integral part of the Indian traditional diet. The RS content in these products varied from 0.81 g % in *khandvi* to 1.50 g % in yellow *dhokla* (Figure 2) and these values were very close to the RS values obtained for one portion of *zunka* and *dhirda* (Figure 1). Thus RS content of different products made from the same basic ingredient may be similar if the amount of ingredient used in the preparation is same and the processing treatment is similar.

In this study, potato *vada* showed RS content similar to the RS in bengal gram flour based products. Surprisingly RS in 100 g of *medu vada* prepared from black gram *dhal* was much lower (Figure 2) as compared to RS in one portion of plain pressure cooked black gram *dhal* (Figure 1). *Medu vada* is a fried snack and studies have reported decreasing effect of both, deep and shallow frying, on RS content<sup>29,30</sup>. Fermentation process may also have a decreasing effect on RS content of foods<sup>31</sup>. Batter for *medu vada* is usually made by partially fermenting soaked and ground black gram *dhal* which might have improved the digestibility of starch and reduced the RS content in *medu vada*.

In the current study, legume preparations were categorised (Table 3) as plain pressure cooked *dhal* category which included four types of *dhal* and 'bengal gram with spinach' preparation, category of *aamti* which included four types of *aamti*, *usal* category which included eight *usal* preparations, category of bengal gram flour products which included *zunka* and *dhirda* and a category of readymade snacks. The foods, in this study, were categorised based on the type of preparation rather than based on types of legumes, considering that an individual, over a period of time, would consume a variety of *dhal* or legumes as *aamti* or as *usal*, rather than a single legume type.

There was no significant difference ( $p \geq 0.05$ ) in the mean RS content of one portion of any of the categories of legume preparations. Though, as

discussed earlier, the RS range for *aamti* was lower than the RS range for plain pressure cooked *dhal*, the mean RS per portion of these two categories was not significantly different (Table 3). This could be because both these categories included same varieties of *dhal* and the quantity of *dhal* per portion was also same (30 g). When categories of bengal gram flour preparations were compared, the mean RS content ( $1.205 \pm 0.04$  g) in one portion of bengal gram flour preparation was not significantly different from mean RS ( $1.168 \pm 0.28$  g) in one portion of readymade bengal gram flour based snacks. Similarly, presence of green gram and bengal gram in all the three categories i. e. plain pressure cooked *dhal*, *aamti* and *usal* may be responsible for not finding significant differences among the mean RS content of these categories. Thus the result once again suggested that the type and the quantity of raw ingredient used in a preparation could be major factors affecting the RS content of any preparation.

### Conclusion

The results of the study generated useful data with respect to RS content in 100 g legume preparations which was in the range of 1.21 g to 2.16 g for pressure cooked *dhals*, 0.42 g to 0.69 g for *aamti*, and 0.70 g to 2.38 g for different *usal* preparations. Based on the RS content of one portion, red gram *dhal*, Bengal gram (*kabuli* and brown) and lentil preparations were identified as preparations with relatively higher RS content. The results also suggested that the RS content of different types of preparations may be similar if the type and quantity of legumes used in these preparations is same.

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