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Hypothesis

A Computational analysis on Lectin and Histone H1 protein of different pulse species as well as comparative study with rice for balanced diet

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Abstract:

The issue of balanced nutrition is of great concern to human. Meat and fish are the best sources of protein. The affordability of these resources for people in developing countries is less. Thus, there is an increasing interest in pulses and its derivates as an alternative to fish and meat. Lectin and histone H1 are the most common proteins in various pulses and our interest is in identifying the dominant essential amino acids in them for use as supplements. However, actin and lectin are common among *Oryza Sativa* and *cicer arietinum*. We describe the amount of lectin and histone H1 in *cicer arietinum*, *Lens culinaris* and *Pisum sativum* in a comparative manner. *cicer arietinum* was found to contain more essential amino acids than *Lens culinaris* and *Pisum sativum*. The secondary structures of lectin and histone H1 protein were analyzed to gain functional inferences in these species. The comparative study shows the relatively poor presence of the amino acid methionine in most pulses. However, *Oryza Sativa* was found to contain sufficient methionine. The study shows that pulses (especially *cicer arietinum*) were a suitable alternative source to meat and fish for Lectin and Histone H1 balance. Hence, pulses could be suggested with rice for balanced protein diet.

Keywords: Lectin, actin, histone H1 protein, balance diet, human, sequence analysis.

Background:

Proteins are the most abundant biological macromolecules occurring in a cell and all parts of cells. They are the chief actors, said to be carrying out the duties specified by the information encoded in genes **[1, 2]**. Needed by the human body importantly for growth and maintenance, there are different sources of proteins. Among them, pulses are important cereals due to their high protein and essential amino acid content **[3]**. Pulses are used as food and animal feed and provide protein, complex carbohydrates and several vitamins and minerals. Like other plant based foods, they contain cholesterol and little fat or sodium. They are 20-25% protein by weight, which is double the protein content of wheat and three times that of rice. Although pulses are generally high in protein

content and the digestibility of that protein is also high, they often are relatively poor in the essential amino acid methionine [3, 4]. In South *Asia* especially in Indian subcontinent, people are habituated to take rice and pulse as their daily meal [4, 5].Rice is the staple food of over half the world's population. It is the predominant dietary energy source for the countries of Asia and the pacific region, 9 countries of North and South America and 8 countries of Africa. Rice provides 20% of the world's dietary energy supply. It is a good source of protein and carbohydrates that are required for energy [5]. Essential amino acids are those that are mandatory for good health but cannot be synthesized by the body and so must be supplemented through the diet. There are ten amino acids generally considered essential for humans [6, 7]. However, rice and pulse are good sources of these essential amino acids [7]. If

the types of pulses or rice which provide more essential amino acids could be found, it will be easy to fulfill our daily nutrients requirements [8]. A comparative study was performed among cicer arietinum, Lens culinaris and Pisum sativum to understand which supplies more essential amino acids. In order to analyze essential amino acid compositions, we considered two common proteins e.g. - Lectin and Histone H1 from cicer arietinum, Pisum sativum, and Lens culinaris. Computational analysis showed that Lectin and Histone H1 are relatively poor in the essential amino acid methionine. The main purpose of our study was to observe the essential amino acids we can identify from pulses and if it is lacking, to provide sufficient essential amino acids that can fulfill our daily essential amino acid requirements. Apart from this, another study was done to see how many essential amino acids are obtained from Oryza sativa Lectin protein or if it provided sufficient amino acids especially Methionine. In order to maintain a balanced diet which will provide more essential amino acids [9], the amino acid compositions and their secondary structures were compared [10].

Methodology:

Sequence Retrieval

The amino acid sequence of lectin, histone H1 and actin from 3 pulse species including *Lens culinaris*, *Cicer arietinum* and *Pisum sativum* and a rice species *Oryza sativa* indica were retrieved from the national center for biotechnology information (NCBI) resource **[11]** with the following accession numbers AAS55887.1, AA062538.2, AAA33675.1 for lectin protein of *Lens culinaris*, *Cicer arietinum* and *Pisum sativum* respectively and AAK29456.1, CAA07233.1, AAA50303.1 for histone H1 protein of *Lens culinaris*, *Cicer arietinum* and *Pisum sativum* respectively. The sequence of *Cicer arietinum* and *Oryza sativa* indica have

accession number as ACD37723.1, CAA33874.1 for actin and AA062538.2, AAD27889.1 for lectin successively.

Analysis of amino acid composition

To evaluate the amino acid composition and physiochemical properties, ProtParam was used. It is available on ExPASY **[12]**.

Multiple sequence alignment

The amino acid sequence of lectin and histone H1 from different pulse species were aligned along with each other using Clustalw2 [13].

Secondary structure analysis

GOR was used to compare and analyse the secondary structure of amino acid sequences. **[14].**

Results:

Sequence analysis from gene bank showed that the protein lectin contains 268 amino acids in *cicer arietinum* whereas it contains 229 amino acids in *Lens culinaris*, and 265 amino acids in *Pisum sativum*. According to the ProtParam result, lectin protein of *Lens culinaris* contains 48.9% of essential amino acids, *cicer arietinum* contains 52%, and *Pisum sativum*, 52.1% essential amino acids (Figure 1B) respectively. In case of histone H1 protein (Figure 3) *Cicer arietinum* comprised of 188 amino acids, *Lens culinaris* contains 293 amino acids and *Pisum sativum* contains 185 amino acids. Protparam indicated that the percentage of essential amino acids for histone H1 protein was 40.9% in *Lens culinaris*, 55.3% in *cicer arietinum* and 55.2% in *Pisum sativum* (Figure 1A).



Figure 1: ProtParam result for amino acid composition of (A) histone H1 protein of *Lens culinaris, cicer arietinum* and *Pisum sativum* (B) lectin protein of *Lens culinaris, cicer arietinum* and *Pisum sativum* (C) lectin protein of *cicer arietinum* and *Oryza sativa* (D) actin protein of *cicer arietinum* and *Oryza sativa*.

The length of amino acid sequences and percentage of essential amino acids are found to be similar between *Pisum sativum* and

cicer arietinum than *Lens culinaris*. Analysis of lectin and histone H1 protein among these three species of pulses using Clustal W2 showed the changes in amino acid sequences and their

multiple sequence alignment (shown respectively in Figure 2 and 3).

	10	20	30	40	50	60	70	80	90	100
	1	1	1	L	1	1	1	1	1	I
lens1_229					GYTTKG	KLTLTKAVKS-	TVGI	RALYSTPIHIN	VDRDTGNVANH	VISETE
Sec.Cons.				ccccccc-	c??ccc	ceeeeecccc-	c??	eeeccceeee?	e?ccccccce	eeeeee
cicer1_268	MISFYLIFLSILLT	TIFFFKVNST	TETTSFSITK	(FSPDQKNLIF	QG-DGYTTKG	KLTLTRAVKN-	TVGI	RALYSSPIHIN	DRETGNVAN	TYTSFTF
Sec.Cons.	ccch????hh????	?eeeeecccc	cccc?eeeec	cccccc?eee	e?-ccccccc	eee??h???c-	ccci	eeeccceeee?	eccccccce	eeeeee
pisum1_265	MALYRTKELVSLVS	IMFVLLATNI	IEALSFNFPK	(ITPGNTAITL	QGNAKILANG	VLALTNSTQIE	PTTTFPSTG	RALYSTPVPL	VDSATGNVASH	TSFSF
Sec.Cons.	ccccccceeehhhh	hhhhhhhhh	hhh??cccc	cccccceeeee	ecch????cc	eeee?cccccc	ccccccccc	eeeccccccc	ccccc?eeee	ee?eee
	::* *:**.:: :.*****:*: :** *****:*:									
Prim.cons.	M2222222L22L22	22F2222222	2E22SF222K	22PG33N3I2	QGN2GYTTKG	KLTLTSAVKSE	PTTTFPTVG	RALYSTPIHIV	VDR3TGNVANE	VTSFTF
	110	120	120	140	150	160	170	190	100	200
	110	120	150	140	150	100	170	100	150	200
leng1 229	VIDAPSSYNVADEE	TEFTAPUDTE	POTGGGY	LOVENSKEYD	KTSOTVAVEE	DTFYNAAWDPS	NKERHIGID	NSTRSVNTR	WNLONGERAN	JUVIAFN
Sec. Cons.	eeccoccccccccc	eeeeecccc	cccccc?	eeeecccccc	cccceeeeee	200000000000000000000000000000000000000	cccceeeee		Percences?	PPPPPP?
cicer1 268	VINAPNSYNVADGE	TEFIAPVDT	POTGGGY	LGVENSAEYD	KTTOTVAVEF	DTFYNAAWDPS	NRDRHIGID	NSTRSVNTKS	WKLONGEEAN	IVVIAFN
Sec.Cons.	ee?cccccccccc	eeeeecccc	cccccc?	eeeecccccc	ccc?eeeeee	200000000000000000000000000000000000000	ccccee???	2000220220	eecccccce	eeeeec
pisum1 265	VILNPSGRVPTDGL	VFFIAPPDTH	TIPNNSOSOY	LGVVDSKT	SINRFVGLEF	D-LYANSFDP-	-YMRHIGID	INSLISTRTVE	RYNFVSGSLTH	WTIIYD
Sec.Cons.	eeeccccccccccc	eeeeccccc	cccccccc	eeeecccc	cccccccee	e-ccccccc-	-ccc??????	?cccccceee	eeeccccce	eeeeec
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Prim.cons.	VI3APSSYNVADGFTFFIAPVDTKPQTGGGSQYLGVFNSKEYDKT3QTVAVEFDTFYNAAWDPSN33RHIGIDVNSIKSVNTKSWNLQNGE3ANVVIAFN									
	210	220	220	240	250	260	270			
	210	220	230	210	200	200	270			
leng1 229	AATNULTUTLTYPN	SLEFFNVTS	TINEVUPIK	DVVPFWVPTC	FSATTGAFFA	AHEVHSWSEHS	FLOOTSSSK			
Sec. Cons.	CCCCERERERERCCC	CCCCCCCCC	2002222000	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	2cccc22hhh	hhhcceeee?	CCCCCCCCCCC	CCCC		
cicer1 268	AATNVLTVSLTYPN	SLEEENVTS	TLSDVVSLK	DVVPEWVRTG	FSATTGAEYA	AHEVT.SWSFHS	ELSGTSSSK	ADA		
Sec. Cons.	ARTIVELVESTTENELEERVISTEERVISTERVVERVITERVITERVITERVERVESKENDER									
pisum1 265	SPENTLTAVITYENGOISTISONVDLKAVLPKDVSVGFSATS-TIAVSHNIHSWSFTSNLFATTGNTVSOV-									
Sec.Cons.										
7.9.7.5.6.6.7.7.7.7.7.										
Prim.cons.	AATNVLTV3LTYPNSLEEENVTSYTLS3VV3LKDVVPEWVRIGFSATTGAE3AAHEVHSWSFHSEL3GTSSSKQAADA									

Figure 2: GOR result of lectin protein for Lens culinaris, cicer arietinum and Pisum sativum.

Secondary structure analysis was also done with GOR to compare secondary structural changes in these significant positions of amino acids changes (Also in Figure 2 & 3). In count of total food value, whole, dried, boiled cicer arietinum contains 27.4gm of carbohydrates, 9.5gm of protein, 2.5gm of lipid, per 100gm, whereas Pisum sativum contains 25.2gm carbohydrate, 9.2gm Protein, 1gm fatty acid per 100gm dried pea. 100gm of dried, boiled Lens culinaris contains 9gm protein, 20gm carbohydrates and 0.4gm of fatty acid. cicer arietinum and Oryza sativa indica were compared in terms of lectin and actin that were common in both. cicer arietinum contains 268 amino acids and Oryza sativa indica contains 200 amino acids for lectin. Both contain 377 amino acids for actin protein. Comparative analysis of essential amino acid composition with ProtParam indicated that actin of cicer arietinum contains 48.2% of essential amino acids, while that of Oryza sativa indica contains 47.8% (Figure 1D). Further, lectin protein contains 52% essential amino acid in cicer arietinum and 21.5% in Oryza sativa indica (Figure 1C). Computational analysis showed that Lectin and Histone H1 were relatively poor in the essential amino acid such as methionine, however, Oryza sativa provided sufficient amino acids especially methionine. Analysis of secondary structure, changes in amino acid sequences and multiple sequence alignment with GOR was done for actin and lectin protein of *cicer arietinum* and *Oryza sativa* indica.

Discussion:

With reference to the analysis of pulse proteins, lectin and histone H1 are good sources of essential amino acids. ProtParam results from the study (Figure 1A & 1B) show the composition of essential amino acids of lectin and histone H1 among cicer arietinum, Lens culinaris, Pisum sativum. It also shows that Lens culinaris provided less number of essential amino acids than cicer arietinum and Pisum sativum (Figure 1A & 1B). It also showed that the other two types of pulses e.g.; cicer arietinum and Pisum sativum provided more essential amino acids than Lens culinaris (Figure 1A & 1B). One of the main aims of our experiment was to observe the amino acids composition among three types of pulses. Accordingly, ProtParam results indicate that Lens culinaris did not provide many essential amino acids as compared to cicer arietinum and Pisum sativum (Figure 1A & 1B). It can thus be said that cicer arietinum and Pisum sativum is better than Lens culinaris as they provide more essential amino acids (Figure 1A & 1B).

A comparison between *Pisum sativum* and *cicer arietinum* showed that both were good sources of essential amino acids. In count of total food value, *cicer arietinum* contains more proteins per 100 gm (discussed in results) **[15]**. Similarly, lectin and histone H1 proteins of the three pulses were compared by clustalW2 software to study the similarities among them. This showed that there were more similarities between *Pisum*

sativum and *cicer arietinum*. The secondary structures of two proteins were also analysed by GOR software which helped predict the functions of proteins (Figure 2 & 3).

	10	20	30	40	50	60	70	80	90	100	
	1	1	1	and a second	1	1	1	1	1	1	
cicer1_188		MSTTAAQPKSKKTA			STKKPLSHPTYAEMITEAIVSLKERTGSSQHAITKFIEEKHKDLSJ					TFRKLI	
Sec.Cons.					cccccc	cccccccc?hhhhhhhhhhhhhhh			hhhhhcccch	ihhhhhh	
pisum1_185			MS-TVAQ	MS-TVAQTKPKKTA		AAKKPLSHPTFAEMITEAITSLK			RTGSSQYAITKFIEEKHKDLPPTYRKLV		
Sec.Cons.			cc-cccccc?hhh		hhcccccc?hhhhhhhhhhhhhhhh			ccccchhhhhhhhhh?ccccc?hhhhh			
lens1_293	SEEPTTVAVEQPIAEEQAAVDTLPPVVNESEEPTAKPKKAAKE				PKAKKAPAKPI	RTHPTYEEMV	KDAIVSLKEK	NGSSQYAIAKI	TIEEKQKQLPS	SNFKKLL	
Sec.Cons.	ddddd?eeeddhhl	hhhhhh <mark>cc</mark>		c?hhhhhhc	a???accccc	cccc?hhhhh	hhhhhhcc	cccchhhhhh	hhhhhh <mark>c</mark> hi	ihhhhhh	
				.*.**:*	11111**	:***: **:	.:**.***:	.****:**:**	****:*:*.,	**:	
Prim.cons.	SEEPTTVAVEQPIAEEQAAVDTLPPVVMS2T3AQ3KPKKTAKEPKAKKA3KKPLSHPTYAEMITEAIVSLKERTGSSQYAITKFIEEKHKDLPPTFRKL3										
	110	120	130	140	150	160	170	180	190	200	
	In the second large		1	1	1			1	1	1	
cicer1 188	LLHLKKSVAAGKL	LKKSVAAGKLVKVKGSFKLAPAKSS		PAKSS	VAKPKAAT			APAATKKAKAVTKPAAKAATKP			
Sec.Cons.	hhhhhhh?cccee			ccccccc			chhhhhhhhhhhhhhhhhhhhcc?				
pisum1 185	LLHLKKSVASGKLVRVKSSFKL		7	APAAAK		TAPAKTS		AATKAPKAVTKPSAKTVTKP			
Sec.Cons.	hhhhhh?cccceeeeeccccc		<mark>c</mark> ????c <mark>-</mark>		ccccc?h			hhhccccccccccccccccc			
lens1 293	LVQIKKLVASGKL	KVKASYKLP.	AKSSAPKPAK	(PAASKPKAK	PKAKPAAKSKI	AKPAAKAKPAI	AKAKPAAKAK	PAAKAKPAAKA	KPAAKAKPA	AKTAAVA	
Sec.Cons.	hhhhhhh?cccee	eeeeccocc		0000000000	cccchhhhhh	?hhhhhhhh	hhhhhhhhh	hhhhhhhhh	hhhhhh?hhi	ihhhhhh	
	*:::** **:***	*:**.*:**		** :.		.* .*.:		**.	. * :**:*	**:.: .	
Prim.cons.	LLHLKKSVASGKLVKVK3SFKLPAKSSAPKPAKAPAASKPKAKPKAKPAAKSKAKP3AKAK3A2KAKPAAKAKPAAKA2PAATKKPKAVTKPAAKTATKP										
	210	220	230	240	250	260	270	280	290		
			1		1	1		1			
cicer1 188	KAKAVAKPKSSGK	AQSCNEAENG	AKPKAK-		-TVKTTPVKK	AVAK	T-TKKVPVK	KP-KSVKTPV	KAKK		
Sec.Cons.	hhhhh?ccccccc		cccccc-		-?ecccchhhhhh?-ccccccccc-cccccchh?ccc						
pisum1 185	KAKAAGSRSQSCGKAEAVAKPKAK				SVKATPVKKAVAKKVVKKAKSVKSPAKKV-KSVKTPVKKAKK						
Sec.Cons.	hhhhcdccc	ccchhhhhh	h?hh??-		?cccchhhhhhhhhhhhh???ccc???c-ccccc?hh?ccc						
lens1_293	<mark>KAK</mark> PAAKAKPAAKAKPAAK <mark>A</mark> KPAAKAKPAAKAKPAAKPAKAARTSTRTSPGTRAAAPKPAAKKAAPAK <mark>KAPVKAAAKTAK</mark> SPA <mark>KKA</mark> AAKRGKK										
Sec.Cons.	hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh										
Prim.cons.	KAKAAAK2K223K	AQSC3KAE33	AKPKPAAKAKE	PAAKPAKAAR	TSVKTTPVKK	AVAKKPA22K	A23KK3PVK	K3AKSVKTPV	KAKKKRGKK		

Figure 3: GOR result of histone H1 protein for Lens culinaris, cicer arietinum and Pisum sativum

Our ProtParam results indicate that cicer arietinum provides more essential amino acids than Lens culinaris and Pisum sativum, but it does not provide all the essential amino acids at high concentration such as methionine for our body. In order to maintain a balanced diet providing us with all the essential amino acids that would be better for us. For this reason, we analyzed rice with pulses. Actin and lectin protein are common proteins between cicer arietinum and Oryza sativa (indica). ProtParam results showed that actin and lectin proteins of Oryza sativa (indica) provides a good numbers of essential amino acids which can be the supplement to pulse proteins (Figure 1C & 1D). Histone H1 of cicer arietinum provides 1.1% methionine and lectin of cicer arietinum provides 0.4% methionine whereas actin protein of Oryza sativa (indica) provides 4.5% of methionine and lectin protein of Oryza sativa (indica) provides 1.5% Of methionine (Figure 1C & 1D). Other essential amino acids that cannot be found properly from pulse proteins could be supplemented by rice proteins. As essential amino acids are needed considerably in our body, we can make a balanced diet which will provide all the essential amino acids better [16]. For this purpose, our computational study revealed that cicer arietinum and Oryza sativa (indica) could be a good combination in case of essential amino acids.

Conclusion:

The types of amino-acids in lectin and histone H1 protein were found to be almost similar. *cicer arietinum* contains more essential amino acid than *Lens culinaris* and *Pisum sativum*. Hence, lectin and histone proteins are suggested alternative to meat and fish for balanced diet with rice.

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