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Process Standardization and Quality Evaluation of Horse Gram Milk Beverage

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Abstract

Horse gram being a rich source of protein, calcium, iron, molybdenum, etc helps in treating many diseased conditions including jaundice, gastrointestinal problems, piles and hemorrhagia. It also contains some anti-nutritional factors like tannins, phytic acid, trypsin inhibitor etc. Moreover, the poor cooking quality of this legume makes its use somewhat limited. Due to the pervasiveness of lactose intolerance all around the world, different alternatives of regular dairy milk are becoming popular. Taking this problem into account, an attempt has been made to extract the milk from soaked horse gram. Soaking is a technique which helps to diminish the anti-nutritional factors. In the present study, horse gram was soaked in water for 18 hours and a smooth paste was prepared. It was then diluted with water at varying dilutions (solid : liquid :: 1:4.64-1:11.36), heated at different temperatures (68.18-101.82 ^{0}C) for variable time durations (4.7-55.23). The impact of different variables on the phytochemical (ascorbic acid, antioxidant activity, total phenolics, tannins and oxalate content) and quality (yield, TSS, titratable acidity, protein content, reducing sugars, total sugars, starch content and overall acceptability) characteristics of the horse gram milk extract was analyzed. All the variables under study possessed a significant effect on all the responses either linear, quadratic or interactive terms which was further confirmed with the cluster analysis where no clear cut clustering was observed on the basis of any variable. It was revealed that the optimum conditions for the extraction of milk were 1:7.55 dilution, 77.05 ^{0}C cooking temperature for 32.85 minutes having 0.96 desirability. The developed conditions can be used at pilot scale which will open a new door in non-dairy product industry.

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KEYWORDS *Horse gram, Horse gram milk, non-dairy milk, phytochemical parameters, quality parameters*

INTRODUCTION

Horse gram (*Macrotyloma uniflorum*) is mainly cultivated for animal feed in dry land areas of India, Sri Lanka, Australia, Burma and (Thirukkumar et al. 2014). The common names of this legume are madras, bean and kulat (Morris 2008). It is popularly referred to as poor man's pulse in Southern parts of India where it is used as a vegetable. It is consumed as sprouts in many parts of India (Wang et al. 2009, Sreerama et al. 2012). The poor cooking quality of horse gram makes its use quite restricted. Horse gram is one of the underutilized legumes, the other being cowpea (Sreerama et al. 2012). It is an excellent source of protein, dietary fibre, phytochemicals, energy, iron, molybdenum and certain micronutrients and at the same time it is also low in fat content (Siddhuraju and Manian 2007, Sreerama et al. 2012). The presence of some anti-nutritional factors such as protease, hemagglutinins, tannins, phytic acid, trypsin inhibitor etc. limits the utility of horse gram. Therefore, methods such as soaking, germination, boiling, fermentation etc. help to diminish the effect of these undesirable constituents in order to increase its nutritional quality, acceptability as well as utilization of the legume (Sreerama et al. 2012). It is good for diabetic patients as it is believed to have low postprandial glucose response (Bravo et al. 1998, Siddhuraju and Manian 2007). It has high insoluble dietary fibre and thus plays a role in maintaining intestinal health. It also possesses antioxidant activity as it shows significant effect against various bacterias including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aereus* (Prasad and Singh 2005, Morris 2008). It helps to treat ailments like jaundice, hemorrhagia, piles and many more. It also maintains body temperature during winter season (Ramesh et al. 2011).

Now-a-days, reports suggest increased prevalence of chronic health disease like lactose intolerance all around globe. Therefore, there is an increased trend of the development of non-dairy products for lactose intolerant people. Many alternate sources of dairy milk are available in the market in order to fulfill the nutritional requirements of an individual and at the same time avoid any nutritional deficiencies. Various attempts have been made by different researchers to design and develop lactose-free milk and milk products such as almond milk (Keller et al. 2012, Maghsoudlou et al. 2016), soy milk (Kuo et al. 2014), rice

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milk (Keller et al. 2012, Adewale et al. 2013, Shukla and Jain, 2017), coconut milk (Belewu et al. 2007), oat milk (Önning et al. 1998, Mårtensson et al. 2000), lactose- hydrolyzed milk (Jelen et al. 2003, Choi et al. 2007) etc. Out of all, soy milk stands out as the best alternative for regular dairy milk (Paul et al., 2019). Gatade et al. 2009 prepared soymilk by soaking soybean in water overnight followed by autoclaving. Then it was washed with warm water and then with cold water. After that, beans were ground with water (1:1) and filtered (2-3 times) to obtain the final product. Similarly, legume-based milk can also be an alternative for the regular dairy milk and a variety of the legumes has been explored for the extraction of milk but the utilization of horse gram for the extraction of milk is still a lacking point in the non-dairy milk industry. Therefore, in the present study an attempt has been made to prepare and evaluate the milk extract from soaked horse gram in order to explore this underutilized legume.

MATERIAL AND METHOD

Horse gram seeds were procured from the local market (Phagwara, Punjab, India), soaked in water for 18 hours and later used for milk extraction. Extra amount of water was drained out. Using a grinder, the soaked seeds were ground to make a smooth paste. The paste (20 g) so obtained was diluted with water at different dilution (solid : liquid ratio :: 1:4.64-1:11.36) followed by heat processing at different temperatures (68.18-101.82 ^{o}C) for different time durations (4.77-55.23 minutes). The filtrate of the respective run was filtered using muslin cloth and packed in the glass bottles, stored at refrigerated temperature and used for further physico-chemical and sensory analysis.

Effect of variables such as water concentration or dilution (A), temperature of cooking (B) and time of cooking (C) on physico-chemical and sensory quality of extracted milk was found by total 20 runs were designed and tested using response surface methodology (RSM). The central composite design (CCD) consisted of three factors i.e., dilution, temperature and cooking time; at 5 levels was done to work out most significant and higher-order effects of different factors with their relationship. Design constitutes of 6 center points and alpha value of ± 1.68. Five coded levels of alpha, studied involved -2, -1, 0, +1 and +2. The lowest and highest ranges of the variables with coded and actual values are given in Table 1. Mathematical relationship of responses (yield, TSS, titratable acidity, ascorbic acid, antioxidant activity, total phenolics, tannins, oxalate, protein content, reducing sugars, total sugars, starch content and overall acceptability) and variables was approximated by a quadratic model equation. Design Expert® version 7.0 (Stat Ease, Inc, Minneapolis, USA)

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generated the polynomials and the contour plots represented in the study. Response value presented are the average values of triplicate experiments.

Statistical Analysis

Statistical software Design Expert® (Stat Ease, Inc, Minneapolis, USA) was used for regression analysis and to find the working parameters for generation of polynomials and the contour plots. Second-order polynomial equation was formulated on the basis of ANOVA while, optimum variables were standardized using Design-Expert optimization toolbox. Cluster analysis was performed by SPSS 16.0 software to suggest comparative and comprehensive overview of physico-chemical and sensory analysis. The output was plotted in form of a dendrogram with interpretation of data accordingly.

Model Validation

The mathematical model generated during RSM implementation was validated by conducting check point studies. Experimentally obtained data were compared with the predicted one and the prediction error was calculated.

RESULTS AND DISCUSSION

The impact of various variables on the functional and quality characteristics of the horse gram milk extract was analyzed. The yield, TSS, titratable acidity, ascorbic acid, antioxidant activity and phenolics ranged from 36.90 to 92.86 ml, 1.70 to 3.50 ^{0}Brix, 0.12 to 0.34 %, 2.79 to 6.61 mg/ 100 g, 25.22 to 59.75 % and 8.63 to 20 GAE mg/100 g respectively. The tannin and oxalate content varied from 22.65 to 46.80 mg/100 g and 38.39 to 82 mg/100 g. The protein content ranged from 16.05 to 38.68 g/100 g, reducing sugar from 0.79 to 2.03 g/100 g, total sugars from 1.35 to 2.72 g/100 g, starch from 0.67 to 1.79 g/100 g and overall acceptance from 4.10 to 7.50 (Table 1).

All main, linear, quadratic and interactive effects were determined for each one and quadratic response surface models were chosen for statistical analysis of all the responses. The precision was determined by using F- ratio, mean, standard deviation, coefficient correlation and lack of fit test. The data obtained for the various responses under study was interpreted and it was known that the R^2 value was more than 70% while lack of fit was highly non- significant. All the values for regression coefficient and correlation coefficient of all the responses ranged between 0.74 and 0.96 (Table 2).

Effect of variables on phytochemical parameters

The functional parameters were ascorbic acid, antioxidant activity, total phenolics, tannins and oxalate content. Effect of dilution at linear and quadratic levels was found to be

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the most significant parameter affecting all the five variables viz. ascorbic acid, antioxidant activity, total phenolics, tannins as well as the oxalate content. Also the temperature of cooking significantly affected the ascorbic acid content and antioxidant activity of soaked horse gram milk at quadratic terms.

A decrease in ascorbic acid content with an increase in temperature and time of cooking was found (Figure 1a) which might be due to the leaching of ascorbic acid into cooking water and getting degraded due to the high temperature and prolong heating of the milk (Lešková et al. 2006). With increase in dilution there was a decrease in ascorbic acid content as well as antioxidant activity (Figure 1a) due to blending of milk with high amount of solvent i.e., water in present case (Austria et al. 1997, Yaqin et al. 2005). The antioxidant activity initially increased with an increase in cooking time and temperature as it totally depends on pigments like carotenoids, anthocyanins (Rice-Evans et al. 1995), ascorbic acid (Kapasakalidis et al. 2006) and total phenol content (Wojdyło et al. 2009) so dependent on these and the reason of the same has been discussed in the phenolics and ascorbic acid content. Further increase in temperature caused decrease in antioxidant activity, which might be due to the degradation of phenolic compounds and phytochemicals at high temperature.

A gradual increase in phenolic extraction was observed with an increase in extraction temperature (Figure 1a) (Jackman et al. 1987, Dai and Mumper 2010) which might be due to the softening of tissue which is responsible for weakening of phenol-protein and phenol-polysaccharide interaction in plant materials and thus accelerated the mass transfer resulting in more extraction of phenolics (Shi et al. 2003, Hemwimon et al. 2007, Silva et al. 2007, Al-Farsi and Lee 2008, Wang et al. 2008). But a further rise in temperature resulted into decrease in the total phenolics due to its oxidation and degradation (Dai and Mumper 2010).

With increase in dilution, there was a decrease in the tannin content due to saturation of solute with high amount of solvent (Figure 1a). But with increase in cooking temperature, an increase in tannin content was observed which might be due to diffusion of contents of molecules in water from the seed making them more available. Similar results have been reported earlier by Shi et al. 2003, Hemwimon et al. 2007, Silva et al. 2007, Al-Farsi and Lee 2008, Wang et al. 2008. Also with increase in dilutuion time, there was decrease in oxalate content of soaked horse gram milk and similar result has been shown by Yaqin et al. 2005 which is due to a static effect that is the saturation of the extract with high amount of solvent in the solution.

Effect of variables on quality parameters

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The physico-chemical parameters selected were yield, TSS, titratable acidity, protein content, reducing sugars, total sugars, starch content and overall acceptability. The yield was significantly affected by dilution and cooking temperature at linear level. TSS was affected significantly at linear and quadratic level by dilution and cooking time whereas titratable acidity was affected by dilution at linear level. Temperature and time were found to significantly affect the protein content and total sugars. The total sugars were also affected linearly and quadratically by dilution. Whereas interaction between dilution and cooking temperature as well as between dilution and cooking time was also found to be significant in case of protein content. The parameters including reducing sugar and starch were significantly affected by dilution and cooking time at linear and quadratic terms as well as cooking temperature at quadratic terms only. And also temperature and time of cooking affected the overall acceptability at quadratic terms.

With increase in dilution rate, there was a sharp rise in yield (Figure 1b) that might be due to softening of the outer layer, accelerating the diffusibility of the molecules and penetration of the solvent into the solid and thus finally increasing the yield (Radhika et al. 2017). A decrease in acidity with an increase in dilution was found (Figure 1b) which might be due to the saturation of the extract (Yaqin et al. 2005) with solute in the solution. With increase in dilution, there was a decrease in the protein content while, with increase in cooking time and temperature there was slight increase in protein content (Figure 1b). Cooking at high temperature for long time results in increase in protein content as due to heat there is denaturation of protein and reduction in digestibility as same has been reported by Orhevba 2011. There was decrease in reducing sugar with the increase in dilution which might be due to dilution of the sugars with increase in concentration of water (Figure 1b) and thus responsible for a gradual decrease. Whereas, with increase in temperature of cooking there was decrease in reducing sugar, total sugars and starch (Figure 1b); similar results were also observed in case of TSS because sugars are the major component representing the TSS and the same has been discussed above in TSS. With increase in dilution, an increase in overall acceptability was observed but further increase in dilution causes a decline in the same which might be dilution of the solids and ultimately seems to be more watery than milky. In relation to temperature of cooking, there was slight decrease in overall acceptance of horse gram milk.

Numerical optimization

To get the combination of the variables according to the desirability for best treatment combinaation, numerical optimization was made by employing central composite design. The

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desired outcomes for different variables and their responses were selected with different weightages. The software generated optimum conditions i.e. dilution (solid to liquid ratio) 1:7.55, cooking at 77.05 ^{0}C temperature for 32.85 minutes along with 0.96 desirability for extraction of horse gram milk. A difference was worked out between predicted and the actual values for different ttributes to check process efficiency and effectiveness of the experiment.

Cluster analysis of the different variables selected for the extraction of horse gram milk

The data obtained from phytochemical and quality parameters horse gram milk was subjected to rescaled distance cluster analysis to get an overview of the variables and their effect on the responses (Figure 2). It is evident from the figure that there was no exact clustering on the basis of any variables which indicated that each variable under study contributed equally for the attributes of horse gram milk.

Conclusion

The present study on "Extraction and evaluation of milk extract from soaked horse gram" was undertaken to explore the possibility of the utilization of horse gram. Optimized conditions (1:7.55 dilution, 77.05 ^{0}C cooking temperature for 32.85 minutes having 0.96 desirability) will definitely help the processors to utilize the horse gram for extraction of milk which can serve as an alternative to the regular dairy milk and milk based products,.

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Table 1: Treatment combinations with their responses obtained during milk prepration from soaked horse gram

Run	Process variables			Responses												
	Dilution	Temperature of cooking	Time of cooking	Yield (ml)	TSS (^{o}B)	Titratable acidity (%)	Ascorbic acid (mg/100 g)	Antioxidant activity (%)	Phenols (GAE mg/100 g)	Tannin (mg/100g)	Oxalate (mg/100g)	Protein(g/100g)	Reducing sugar(g/100g)	Total sugars(g/100g)	Starch (g/100g)	Overall acceptance
1	8	85	4.77	68.45	2.2	0.25	3.39	30.64	12.30	26.86	48.7	19.04	1.13	1.347	0.9842	4.1
2	4.63	85	30	46.22	3.5	0.34	6.61	59.75	20	46.80	82	28.14	2.03	2.724	1.7915	4.2
3	8	68.18	30	80.36	2.7	0.24	3.11	28.11	11.41	22.65	49.7	17.97	1.48	1.914	1.2947	6.8
4	8	85	30	75.00	3.1	0.2	3.08	27.84	10.58	25.89	52.8	20.69	1.75	1.995	1.5431	7.1
5	8	85	55.22	71.43	3.2	0.27	3.09	27.93	9.48	28.81	51.1	26.19	1.82	2.562	1.6052	7
6	8	101.81	30	36.90	2.9	0.21	2.89	26.12	10.47	30.62	50.4	29.67	1.62	2.643	1.4189	6.9
7	6	75	45	77.78	3.1	0.32	3.99	36.06	13.27	33.47	66	21.8	1.75	2.238	1.5431	6.5
8	8	85	30	75.00	3	0.22	3.69	33.35	12.38	27.98	53.6	24.06	1.69	2.076	1.481	7.3
9	8	85	30	75.00	3.1	0.25	3.79	34.26	13.01	28.54	47.7	23.8	1.75	2.076	1.5431	7.2
10	8	85	30	75.00	3.2	0.23	3.59	32.45	12.89	27.60	49.1	21.4	1.82	1.914	1.6052	7.4
11	6	75	15	88.89	2.8	0.29	4.09	36.97	9.70	30.52	69.13	25.67	1.55	2.4	1.3568	7
12	10	95	15	82.86	2.1	0.19	3.09	27.93	8.63	23.15	45.65	23.73	1.06	2.076	0.9221	5.4
13	8	85	30	75.00	3.3	0.26	3.69	33.35	10.93	27.76	48.8	20.53	1.89	1.914	1.6673	7.2
14	6	95	15	73.02	3.2	0.29	2.79	25.22	15.63	30.81	68.3	25.67	1.69	2.4	1.481	6.5
15	8	85	30	75.00	3.2	0.24	3.6	32.54	12.45	28.00	50.4	23.13	1.82	1.914	1.6052	7.5
16	10	75	15	92.86	2	0.19	4.09	36.97	9.86	24.08	44	16.05	1	1.59	0.86	5.4
17	6	95	45	71.43	3	0.3	3.09	27.93	14.20	32.31	66.1	19.20	1.82	2.562	1.6052	5.5
18	11.36	85	30	72.50	1.7	0.12	3.69	33.35	11.91	22.84	38.39	22.36	0.79	2.076	0.6737	4.6
19	10	95	45	80.00	2.5	0.18	2.79	25.22	11.06	22.93	46	38.68	1.48	2.4	1.2947	5.2
20	10	75	45	89.05	2.6	0.19	3.6	32.54	9.88	22.89	49.5	25	1.41	1.804	1.2326	5

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Table 2: Anova and regression coefficient of the second order polynomial model for the response variables

Variable	DF	F value											
		Yield	TSS	Acidity	Ascorbic acid	Antioxidant	Phenols	Tannins	Oxalate	Protein	Reducing sugar	Total sugar	starch	Overall acceptance
Model	9	6.89	19.08	0.052	4.78	4.78	3.72	7.71	26.37	7.48	22.57	6.97	22.57	3.21
A	1	54.29*	102.26*	175.11*	20.15*	20.15*	18.60*	55.88*	206.85*	0.023	114.23*	17.30*	114.23*	1.69
B	1	6.60*	1.18	1.69	0.78	0.78	0.70	1.37	0.016	18.25*	2.44	15.07*	2.44	0.15
C	1	0.084	22.52*	1.05	0.045	0.045	7.158E-004	0.40	0.17	8.09*	39.83*	14.49*	39.83*	0.89
A^2	1	0.022	22.14*	5.071E-004	7.99*	7.99*	6.67*	8.30*	28.23*	3.25	25.09*	8.30*	25.09*	20.65*
B^2	1	0.30	9.08*	0.18	5.78*	5.78*	1.94	1.44	0.53	1.03	9.97*	3.97	9.97*	0.17
C^2	1	0.51	14.90*	5.66*	3.16	3.16	2.04	0.36	0.43	0.12	16.68*	0.14	16.68*	6.79*
AB	1	0.045	0.45	0.044	2.97	2.97	2.07	5.483E-006	0.018	12.08*	0.059	2.13	0.059	0.57
AC	1	1.238E-003	4.02	1.11	0.38	0.38	4.308E-003	0.59	1.77	24.67*	2.91	1.07	2.91	0.16

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BC	1	0.061	2.43	0.40	0.12	0.12	0.29	3.948E-003	0.25	0.25	0.059	0.70	0.059	0.018
LACK OF FIT		4039.62	0.20	4.783E-004	2.32	189.26	23.35	68.29	60.52	46.76	0.074	0.30	0.060	6.23
R^2		0.8611	0.9450	0.9489	0.8115	0.8115	0.7701	0.8740	0.9596	0.8707	0.9531	0.8624	0.9531	0.7427
Adj R^2		0.7361	0.8955	0.9028	0.6419	0.6419	0.5631	0.7606	0.9232	0.7543	0.9109	0.7386	0.9109	0.5112
CV %		15.94	5.62	7.02	14.80	14.80	14.10	9.53	5.51	10.30	6.36	8.61	6.55	12.86

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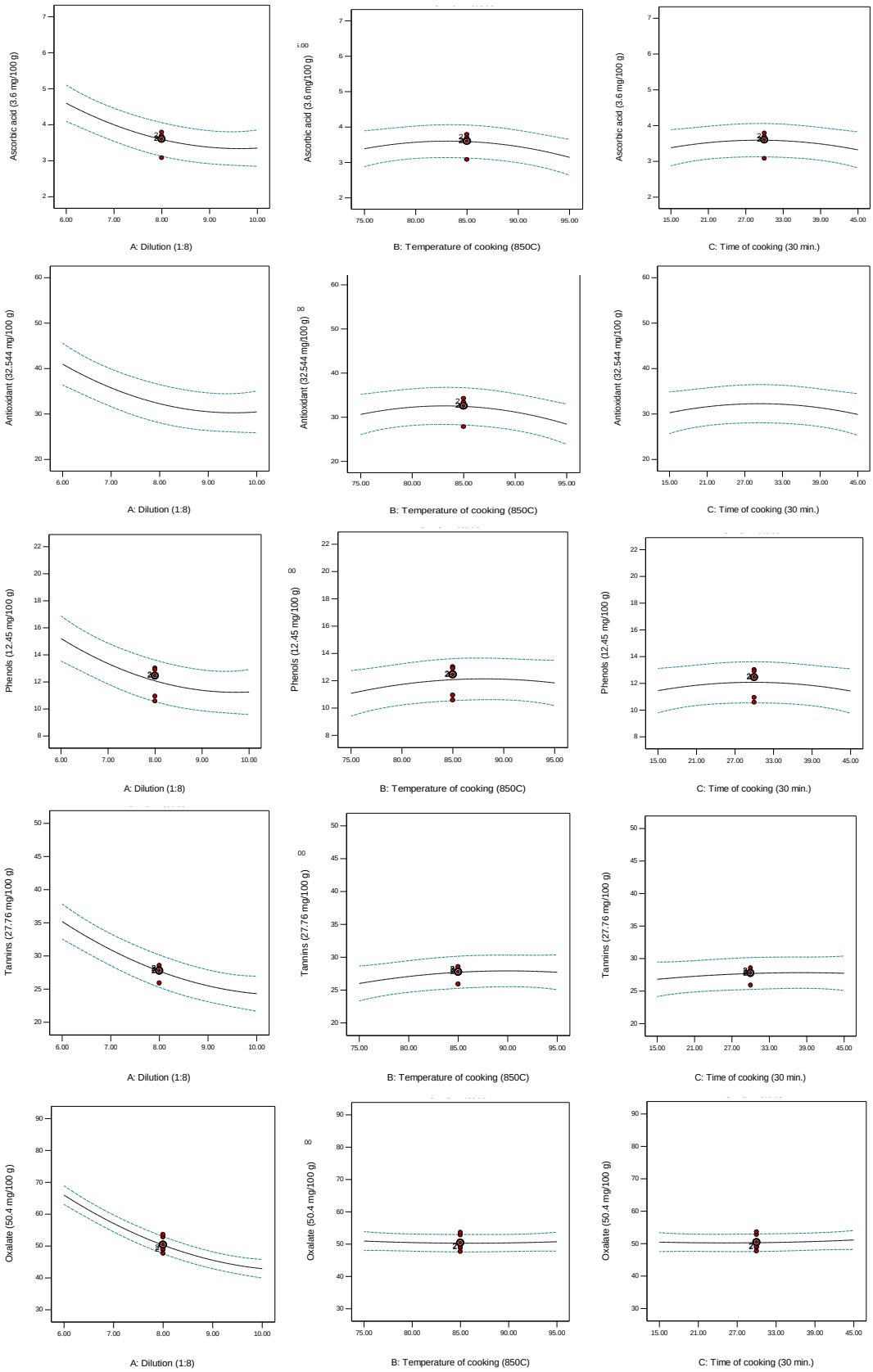

Fig 1a: Influence of each processing variables on phytochemical parameters of horse gram milk

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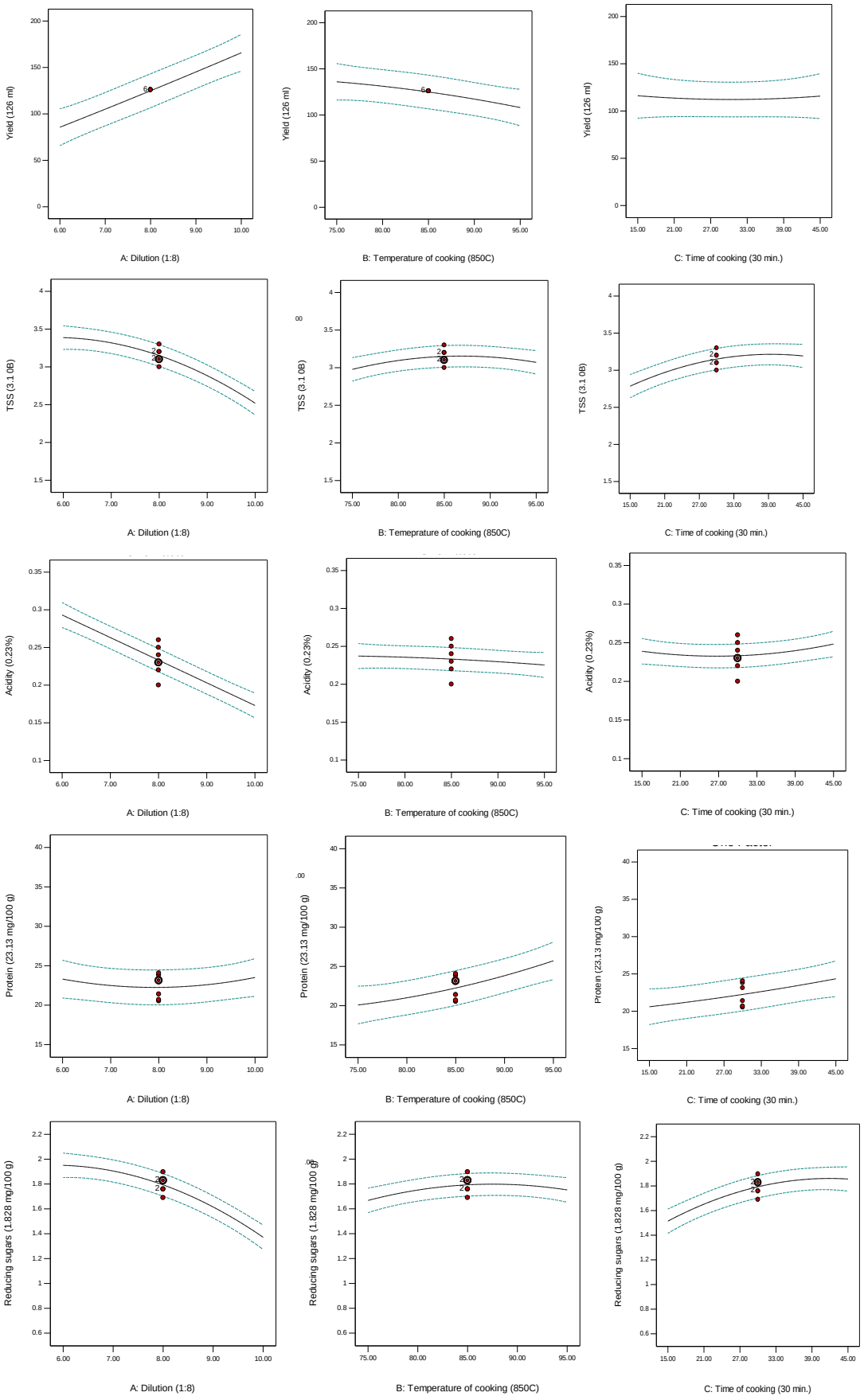

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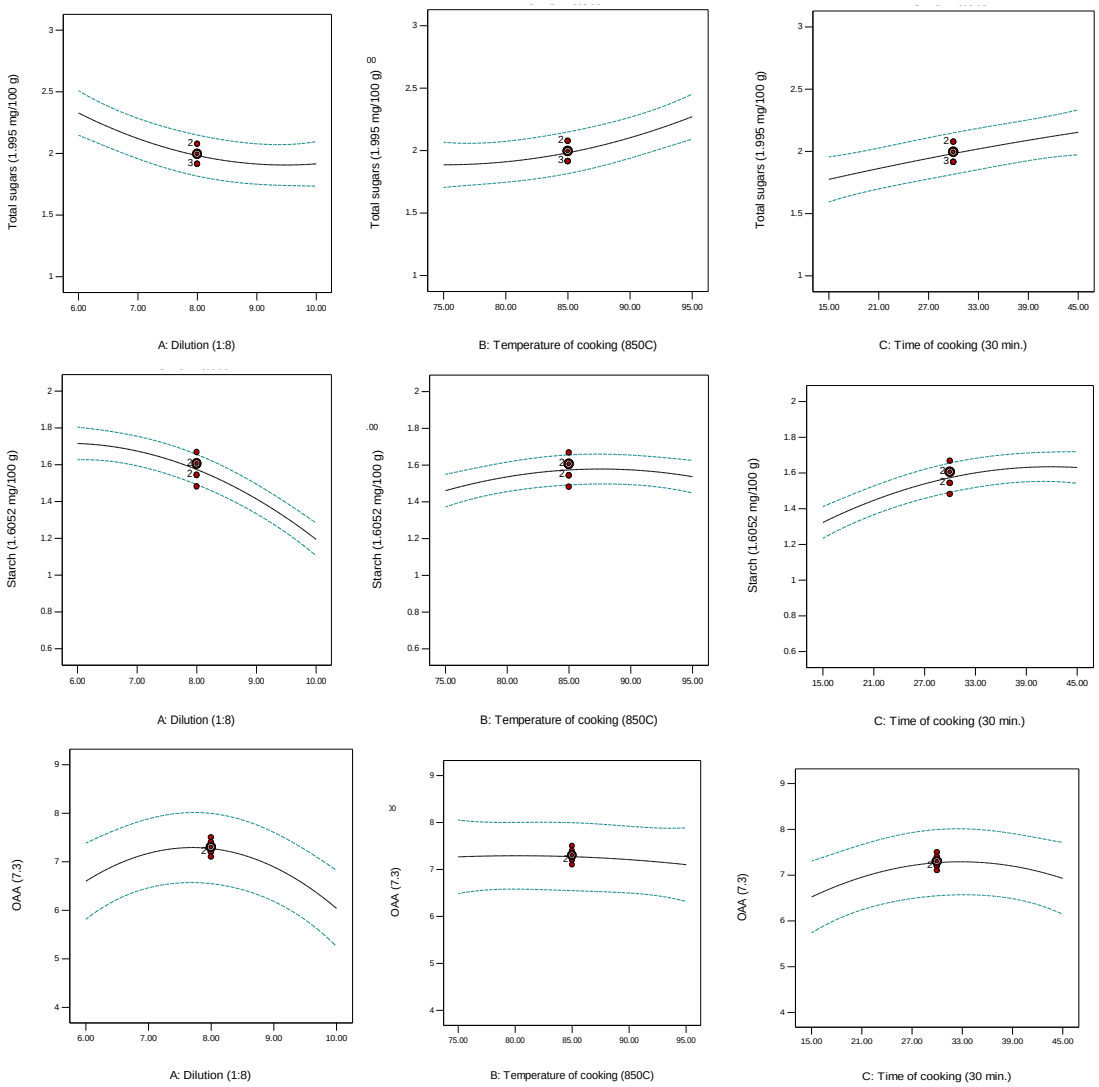

Fig 1b: Influence of each processing variables on quality parameters of horse gram milk

Figure 1: Influence of each processing variables on response variables when other processing variables are held constant; numbers next to X and Y axes labels correspond to the value of each variable at the red dotted point

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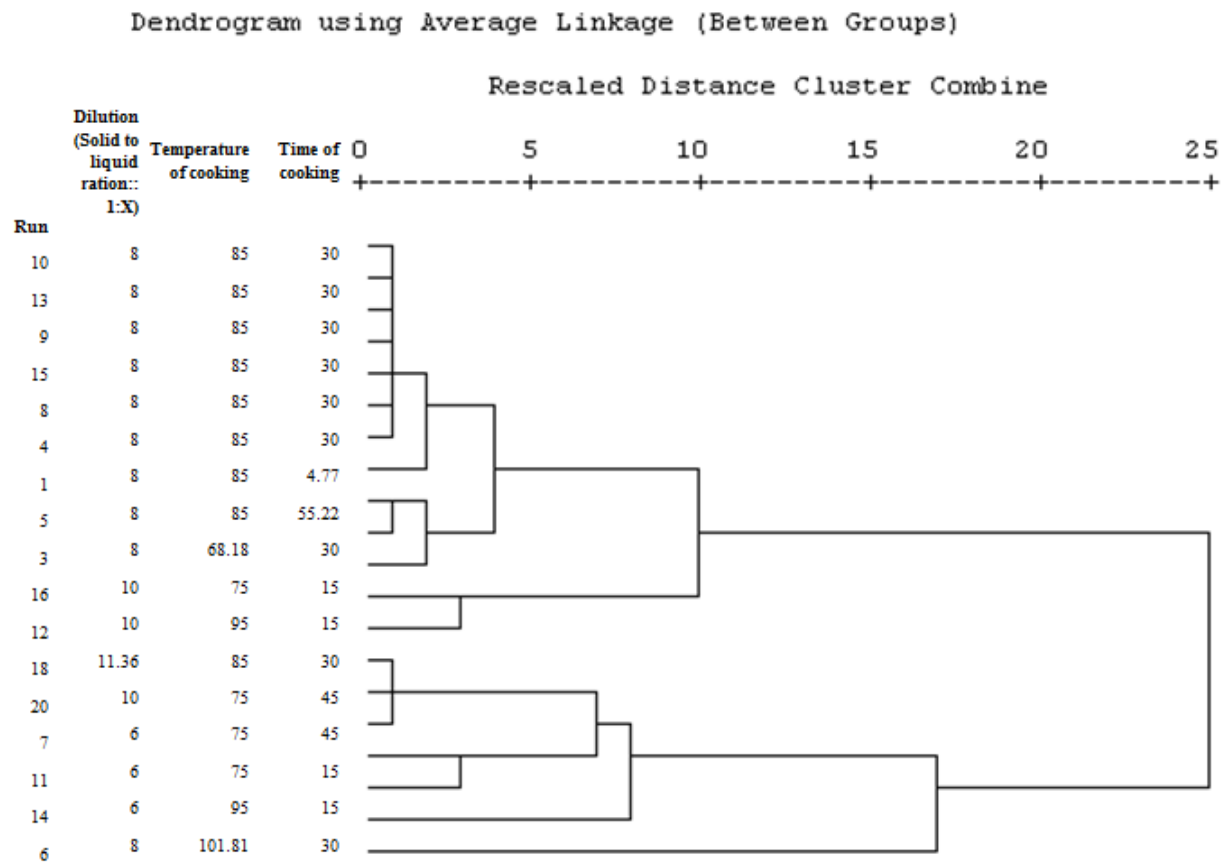

Figure 2: Cluster analysis of the different variables selected for the extraction of **horse gram milk**