Evaluation of amino acid profile of cheddar cheese formulated with xanthan gum and/or sodium caseinate as fat replacers

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***Abstract*—** The aim of the present study was to investigate the effect of incremental reduction of fat contents (in the range of 33 to 6% (wt/wt)) and applying different levels of fat replacers (xanthan gum and sodium caseinate) on amino acid profile and composition of cheddar cheese. Total amino acid contents were determined by using a rapid, accurate, sensitive method. This method includes protein hydrolysis, *o*-phthalaldehyde derivatization, and reversed-phase high-performance liquid chromatography. Amino acid nitrogen (N) increased significantly with decreasing fat content. Decreased fat level resulted in significantly linear increases protein content.

Key words: Cheddar cheese; amino acid; *o*-phthalaldehyde derivatization; fat replacer; xanthan gum; sodium caseinate

Introduction:

Increased awareness of people on health benefits of low-fat diets has led to an increased demand for low-calorie food in particular for low and reduced fat cheeses (Konuklar et al., 2004). Market research reported that increasing future cheese consumption, achieved by focus on produce reduced fat cheeses (Fenelon et al., 2000). Fat content in the cheese is responsible for its many desirable functional, textural, and sensory properties. Low and reduced fat cheddar cheeses typically have poor body and flavor. Different techniques used for improvement of low and reduced fat cheeses include process modifications, using special starter cultures and fat replacers (Mistry, 2001). The use of fat replacers, in particular those of protein or carbohydrate nature in low-fat cheese formulation, has increased due to their easy incorporation into the process and improve the textural properties of reduced-fat cheeses (Lobato-Calleros et al. 1999). Reduced-fat cheddar cheeses have rubbery body and texture due the high level of casein content of it (Mistry, 2001).

Although a well balanced breakdown of casein into small peptides and free amino acids during the ripening is necessary for the development of an acceptable cheddar cheese flavor, there is an inadequate breakdown of casein in low and reduced fat cheeses (Singh et al., 2003; Mistry and Kasperson, 1998). Therefore proteolysis and breakdown of the amino acids are critical events leading to development of texture and flavor in most rennet curd cheese varieties during ripening (Fox and Wallace, 1997). By monitoring the amino acid content as precursors of flavor compounds we are able to find suitable adjunct starter cutlers to increase proteolysis and development texture and flavor defects in reduced fat cheeses.

Amino acid analysis can be performed by using various techniques. Usually, techniques based on ion-exchange separation coupled with post-column derivatization (e.g., with ninhydrin, the "classical" method) are considered more precise than those based on pre-column derivatization and reversed-phase high-performance liquid chromatography (RP-HPLC), because the latter techniques imply extensive sample manipulation before analysis and are affected by the limited stability of the preformed derivatives (Bartolomeo and Maisano, 2006).

The aim of the present study was to describe the effect of incremental reduction in fat content and applying different level of fat replacers (xanthan gum and/or sodium caseinate) on changes of total amino acids. The validation of rapid, accurate, sensitive method was used to determine the amino acids in cheeses contained different level of fat replacers. To our knowledge, this is the first research that shows the effect of using xanthan gum and sodium caseinate as fat replacers on amino acid contents of cheddar cheese containing different fat levels.

Materials and Methods

Preparation of cheddar cheese

For preparation of cheddar cheese, 10 kg milk for each sample was provide from farm of Universiti Putra Malaysia and standardized to obtain the desired fat level base on experimental design. It was mixed with sodium caseinate and xanthan gum purchased from vis Food tech Company (Malaysia, Kualalumpure) according to formulation described in Table 1. The different enriched milks pasteurized at 65 °C for 30 min After stabilizing the temperature at 32 °C ,freeze-dried direct vat set (DVS) mesophilic starter culture (R-704: *Lactacoccus lactis spp. lactis* and *Lactacoccus lactis spp. cremories*) was kindly provided by Chr-Hansen (Hørsholm, Denmark) added (0.015 mL/kg) and allowed to ripen for 20 min. standard cheese rennet was kindly provided by Chr-Hansen (Hørsholm, Denmark) laboratory added (0.026 gr/kg) of milk as suggested by the manufacturer, and the milk mixed thoroughly for 1 or 2 min and Then curd was allowed to set for 45 min and was then cut into approximately 1 cm^3 cubes with vertical and horizontal knives. After being cut, the curd was left to settle for 15 min and stirred gently with a spoon for 5 min .The temperature was increased gradually within 30 min from 30 °C to 39 °C and was maintained at 39 °C ,15

min then the whey was drained, The curd was piled and cheddaring process until pH 5.42. The curd was milled and salted (0.25% w/w).The salted curd was allowed to rest for 5 min after which it was hooped and pressed overnight for 12 h at 2.10 kg/cm^2. The pressed cheese was vacuum packaged in cryovac bags by using a vacuum packaging machine (Model vac master, USA).

Chemical analysis

The concentration of fat in milk, cream and cheese were determined by the Gerber method (James, 1995). Total protein content of milk and cheese samples was determined by measuring total nitrogen using the Kjeldahl method (AOAC, 1997 using method 920.123) and converting it to protein content by multiplying by 6.38.

Reagents, Solvents, and Materials

Sodium phosphate monobasic monohydrate, sodium hydroxide, boric acid, acetonitrile (LC grade), and methanol (LC grade) were obtained from Merck KGaA (Darmstadt, Germany). OPA reagent was prepared as described before (Agilent art. 5061-3335, Palo Alto, CA). Borate buffer was prepared by adjusting 0.4 N boric acid to pH 10.2 with NaOH. HCl was obtained from Sigma-Aldrich (St. Louis, MO). Disposable glass test tubes (50 × 6 mm) and hydrolysis reaction vials (25 × 120 mm) with Mininert valves were from Kimble Glass, Inc., and Kontes Glass Co. (Vineland, NJ). Amber wide-opening vials, glass conical inserts with polymer feet, and screw caps were from Agilent. Amino acid standard mixtures were obtained from Agilent.

Sample preparation for amino acid analysis

In the present study, amino acid content was determined by using reversed-phase high performance liquid chromatography (RPHPLC). Cheeses with different fat levels were weighed based on the percentage of their protein divided by four and then hydrolysed by adding 15 mL 6 mol L−1 HCl to the sample, mixing well in a stoppered test tube for 24 h at 110 ◦C. The hydrolyzed samples were automatically derivatized with OPA by programming the robotic autosampler according to the method described by Bartolomeo and Maisano (2006).

After derivatization, an amount equivalent to 2.5 µL of each sample was injected on a Zorbax Eclipse-AAA column, 5 µm, 150 × 4.6 mm (Agilent), at 40°C, with detection at λ = 338 nm. Mobile phase A was 40 mM NaH_2PO_4, adjusted to pH 7.8 with NaOH, while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v). The separation was obtained at a flow rate of 2 mL/min with a gradient program that allowed for 1.9 min at 0% B followed by a 16.3-min step that raised eluent B to 53%. Then washing at 100% B and equilibration at 0% B was performed in a total analysis time of 26 min.

Experimental design and statistical analysis

A Taguchi design was applied as experimental design with 3 independent variable x_1 (x_1 (fat in cheese milk content), x_2 (xanthan gum content) and x_3 (sodium caseinate content), on 16 type of amino acid as response variable. Independent variable ranges studied were: percentage of fat in cheese milk (1.25-2 -2.75-3.5% w/w), xanthan gum (0- 0.015 -0.030 -0.045 % w/w) and sodium caseinate (0- 0.15-0.30-0. 45% w/w). Therefore, 16 treatments were assigned based on Taguchi design that the following Table (1) is shown. In order to evaluate the interaction effect of significant factors (Fat, Sodium caseinate) on response variable, a full factorial design was used. The data obtained from the measurements were subjected to univariate one way analysis of variance (ANOVA) to determine the significant differences among the sample and values were compared using the Tukey's test defined at $p < 0.05$. Two way analysis of variance (ANOVA) was used to determine the significant or non-significant interaction effect of independent variables on response variable studied. All measurements were carried out in triplicate and reported as the mean ± SD of independent trials. All statistical analysis was performed using the Minitab v. 14 statistical package (Minitab Inc., State College, USA).

Table 1. Levels of independent variables established according to the taguchi design

Group	Run order	Fat in milk cheese	xanthan gum (XG)	sodium caseinate (SC)
LFC	LFC_1	1.25	0.000	0.45
	LFC_2	1.25	0.015	0.30
	LFC_3	1.25	0.030	0.15
	LFC_4	1.25	0.045	0.00
HFC	HFC_1	2	0.000	0.30
	HFC_2	2	0.015	0.45
	HFC_3	2	0.030	0.00
	HFC_4	2	0.045	0.15
RFC	RFC_1	2.75	0.000	0.15
	RFC_2	2.75	0.015	0.00
	RFC_3	2.75	0.030	0.45
	RFC_4	2.75	0.045	0.30
FFC	FFC_1	3.5	0.000	0.00
	FFC_2	3.5	0.015	0.15
	FFC_3	3.5	0.030	0.30
	FFC_4	3.5	0.045	0.45

LFC: Low fat cheese
HFC: Half fat cheese

RFC: Reduced fat cheese
FFC: Full fat cheese

Result and Discussion

Effect of fat replacers on protein content

The average fat and protein content of Cheddar cheese made with different fat levels and fat replacers is summarized in Table 2. There was large Inter-blocks difference in protein content. Reduction of inter-blocks fat level resulted in significantly linear increases in protein. In agreement with the result of this study Guinee et al. (2000) reported that reduction of fat content in milk for manufacture of Cheddar is associated with increasing protein contents (Guinee et al., 2000). The higher protein content results in a more dense protein matrix, this coupled with smaller more uniform fat globules, due to the lower aggregation, results in the more firm and elastic textures commonly found in lower fat cheeses (Mistry and Anderson., 1993). These findings were expected and confirmed the work of other investigators (Brummel and Lee, 1990; Bullens et al., 1994, Sipahioglu.et al., 1999).

Table 2. Chemical composition of cheese 1 day after manufacture

Run order	Response variable	
	fat%	protein%
LFC_1	$7.00^{(a)}$	$35.9880^{(a)}$
LFC_2	$6.50^{(a)}$	$35.4660^{(a)}$
LFC_3	$6.50^{(a)}$	$35.1100^{(a)}$
LFC_4	$7.25^{(a)}$	$34.5665^{(a)}$
HFC_1	$14.50^{(b)}$	$29.7063^{(b)}$
HFC_2	$14.75^{(b)}$	$30.6110^{(b)}$
HFC_3	$14.50^{(b)}$	$29.1210^{(b)}$
HFC_4	$14.75^{(b)}$	$28.4463^{(b)}$
RFC_1	$21.00^{(c)}$	$26.3500^{(c)}$
RFC_2	$20.75^{(c)}$	$26.0650^{(c)}$
RFC_3	$21.25^{(c)}$	$27.5301^{(c)}$
RFC_4	$21.25^{(c)}$	$26.9861^{(c)}$
FFC_1	$28.00^{(d)}$	$23.1750^{(d)}$
FFC_2	$28.00^{(d)}$	$23.4410^{(d)}$
FFC_3	$27.75^{(d)}$	$23.8265^{(d)}$
FFC_4	$28.25^{(d)}$	$24.2405^{(d)}$

Changes of amino acid content based on Taguchi design

The main effect of each factor on the response variable base on Taguchi design is shown in Table 4. The linear effects of fat and sodium caseinate were shown significantly ($p < 0.05$) affected on changes of total amino acid content. Increase of fat content had significantly ($p < 0.05$) affected on decrease of total amino acid content reversely increase of sodium caseinate content had significantly affected on increase of total amino acid content (Table 3). Possible explanation of this result might be due to increase of dense of protein content in cheese concomitant with increase of sodium caseinate content and decrease of fat in cheese. Moreover, no significant difference ($p > 0.05$) was observed on amino acid value by increasing Xanthan gum content. Figure 1 can be supported the data reported in Table 4. The *F* ratio reported in table 4 revealed that the effect of fat was more powerful than sodium caseinate in changes of amino acid content. Arginine is responsible for unpleasant or bitter taste (Pappa and Sotirakoglou, 2008), therefore the concentration of that in low fat cheeses were higher than others. These findings further support the idea of Low fat cheese are more susceptible for the formation of bitter taste.

Changes of amino acid content based on full factorial design

Effect of fat and sodium caseinat as independent variable on 16 type of amino acid base on full factorial design reported in Table 5. The *p*-Val reported in Table 5 evidently shows that linear effect of fat in cheese milk had significantly ($p \leq 0.05$) effect on 16 response variable. Full factorial analysis revealed that the main effect of sodium caseinate had not significant effect on changes of all response variables (Table 5). The main effect of sodium caseinate was not significant ($p > 0.05$) on changes of Glu, Thr, Ala, val, phe, Lys. The effects of sodium caseinate on the rest of amino acids were shown significant effect ($p \leq 0.05$). Moreover the interaction effect of fat in cheese milk and sodium caseinate are shown significantly ($p \leq 0.05$) on changes of all response variables.

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Table 3. Amino acid profile of different fat content of fresh cheddar cheeses

Amino acid	Low Fat Cheese (LFC)				Half Fat Cheese (HFC)			
(Picomol/gr)	LFC_1	LFC_2	LFC_3	LFC_4	HFC_1	HFC_2	HFC_3	HFC_4
Asp	31.7 ± 0.68	30.4 ± 0.81	29.2 ± 0.60	26.0 ± 0.99	24.3 ± 0.06	24.7 ± 0.31	23.8 ± 0.29	24.1± 0.10
Glu	90.1 ± 0.62	88.9 ± 0.47	86.0 ± 1.50	85.2 ± 0.84	81.3 ± 0.20	82.2 ± 0.7	78.4 ± 0.36	79.8 ± 0.78
Ser	51.9 ± 0.24	49.1 ± 0.67	45.3 ± 0.95	43.4 ± 0.54	40.6 ± 0.68	41.6 ± 10	38.9 ± 0.25	40.0 ± 1.11
His	38.0 ± 0.56	35.1 ± 0.78	32.0 ± 0.25	29.0 ± 1.70	27.4 ± 0.36	28.8 ± 0.66	26.0 ± 0.21	26.6 ± 0.09
Gly	48.2 ± 0.81	46.6 ± 0.36	43.6 ± 0.58	41.6 ± 0.47	39.4 ± 0.35	40.3 ± 0.50	37.1 ± 0.27	38.8 ± 0.21
Thr	40.9 ± 0.20	39.9 ± 0.29	38.7 ± 0.42	36.6 ± 0.96	33.1 ± 0.74	34.5 ± 0.32	31.3 ± 0.85	32.4 ± 0.48
Arg	37.8 ± 0.50	36.6 ± 1.01	33.3 ± 0.46	32.7 ± 0.24	31.8 ± 0.07	32.7 ± 0.40	30.9 ± 0.13	31.5 ± 0.14
Ala	28.5 ± 0.35	26.9 ± 0.28	25.6 ± 1.27	25.7 ± 0.45	24.4 ± 0.19	24.9 ± 0.48	23.1 ± 0.53	24.2 ± 0.27
Tyr	25.3 ± 0.31	23.4 ± 0.70	21.7 ± 0.79	20.8 ± 0.17	19.2 ± 0.25	19.6 ± 0.92	18.2 ± 0.42	18.8 ± 0.17
Val	43.2 ± 0.46	42.4 ± 0.33	41.4 ± 0.66	40.3 ± 0.70	38.3 ± 0.17	39.1 ± 0.12	36.3 ± 0.43	37.3 ± 0.67
Met	21.7 ± 0.77	20.1 ± 0.41	18.8 ± 0.11	18.2 ± 0.07	17.5 ± 0.05	17.7 ± 0.11	16.7 ± 0.11	17.2 ± 0.06
Phe	6.6 ± 0.32	5.8 ± 0.06	5.1 ± 0.16	4.7 ± 0.04	4.0 ± 0.41	4.3 ± 0.24	3.2 ± 0.16	3.9 ± 0.19
Iso	27.7 ± 0.70	26.6 ± 0.38	24.9 ± 0.67	24.1 ± 0.09	22.5 ± 0.14	23.5 ± 0.03	21.9 ± 0.23	22.2 ± 0.34
Leu	35.7 ± 0.70	34.9 ± 0.14	33.6 ± 0.25	32.7 ± 0.35	29.9 ± 0.12	31.2 ± 0.34	29.0 ± 0.24	29.6 ± 0.21
Lys	83.1 ± 0.57	80.2 ± 0.88	77.9 ± 0.47	76.8 ± 0.67	73.5 ± 0.60	74.7 ± 0.46	71.9 ± 0.24	72.5 ± 0.49
Pro	113.8 ± 3.79	109.0 ± 0.12	107.3 ± 1.11	97.9 ± 0.60	80.1 ± 0.93	82.4 ± 1.51	73.6 ± 1.38	75.7 ± 2.49

LFC_1: Low fat cheese (0 XG and 0.45 SC); LFC_2: Low fat cheese (0.015 XG and 0.30 SC); LFC_3: low fat cheese (0.030 XG and 0.15 SC); LFC_4: low fat cheese (0.045 XG and 0 SC); $RFC1_1$: Reduced fat cheese (0 XG and 0.3 SC); $RFC1_2$: Reduced fat cheese (0.015 XG and 0.45 SC); $RFC1_3$: Reduced fat cheese (0.030 XG and 0 SC); $RFC1_4$: Reduced fat cheese (0.045 XG and 0.15 SC)

Table 3. Continued

Amino Acid (Pico mol/gr)	Reduced Fat Cheese (RFC)				Full Fat Cheese (FFC)			
	RFC_1	RFC_2	RFC_3	RFC_4	FFC_1	FFC_2	FFC_3	FFC_4
Asp	22.6 ± 0.19	22.2 ± 0.28	23.4 ± 0.30	22.7 ± 0.10	20.5 ± 0.41	20.9 ± 0.02	21.1 ± 0.07	21.6 ± 0.17
Glu	72.8 ± 0.48	72.8 ± 0.12	74.6 ± 0.50	73.7 ± 0.42	65.8 ± 0.33	68.7 ± 0.97	70.3 ± 0.34	71.6 ± 0.07
Ser	36.1 ± 0.92	36.1 ± 0.05	38.2 ± 0.64	37.5 ± 0.58	32.0 ± 0.13	32.9 ± 0.39	33.7 ± 0.30	35.5 ± 0.30
His	25.0 ± 1.69	23.5 ± 0.58	25.7 ± 0.98	25.4 ± 0.76	20.1 ± 0.2	21.1 ± 0.27	21.4 ± 0.04	21.8 ± 0.30
Gly	34.6 ± 0.13	33.0 ± 0.40	36.1 ± 0.16	35.3 ± 0.41	28.8 ± 0.048	29.9 ± 0.29	30.4 ± 0.18	30.9 ± 0.55
Thr	25.8 ± 1.15	24.5 ± 0.18	28.2 ± 0.37	26.9 ± 0.11	21.3 ± 0.17	21.8 ± 0.24	22.5 ± 0.36	24.02 ± 0.11
Arg	28.6 ± 0.09	28.1 ± 0.12	29.8 ± 0.55	29.1 ± 0.01	26.0 ± 0.10	26.8 ± 0.31	27.5 ± 0.03	28.1 ± 0.19
Ala	21.4 ± 0.07	21.0 ± 0.09	22.4 ± 0.41	21.5 ± 0.49	17.6 ± 0.04	18.4 ± 0.36	18.9 ± 0.44	19.8 ± 1.20
Tyr	16.1 ± 0.18	15.6 ± 0.21	17.0 ± 0.34	16.4 ± 0.13	13.2 ± 0.04	13.7 ± 0.08	14.1 ± 0.02	14.6 ± 0.58
Val	32.8 ± 0.12	32.5 ± 0.32	34.2 ± 0.76	33.4 ± 0.23	27.9 ± 0.2	28.9 ± 0.47	30.1 ± 0.15	30.9 ± 0.82
Met	14.2 ± 0.18	13.8 ± 0.11	15.4 ± 0.21	14.7 ± 0.25	11.5 ± 0.2	11.9 ± 0.16	12.4 ± 0.22	12.7 ± 0.09
Phe	2.1 ± 0.17	1.8 ± 0.12	2.9 ± 0.28	2.5 ± 0.07	0.2 ± 0.12	0.49 ± 0.07	0.8 ± 0.13	1.03 ± 0.11
Iso	20.9 ± 0.27	20.5 ± 0.01	21.9 ± 0.11	21.4 ± 0.19	19.1 ± 0.09	19.4 ± 0.29	19.5 ± 0.16	19.9 ± 0.16
Leu	27.1 ± 0.11	26.7 ± 0.25	28.5 ± 0.12	27.6 ± 0.37	24.1 ± 0.15	24.4 ± 0.11	24.8 ± 0.04	25.8 ± 0.13
Lys	70.1 ± 1.00	68.8 ± 0.08	71.4 ± 0.10	70.6 ± 1.18	63.6 ± 0.19	65.2 ± 0.36	65.9 ± 0.19	67.7 ± 0.26
Pro	58.2 ± 0.13	56.5 ± 1.76	69.3 ± 1.56	61.3 ± 1.44	43.1 ± 1.85	46.3 ± 1.32	54.8 ± 0.60	56.6 ± 0.36

$RFC2_1$: Reduced fat cheese (0 XG and 0.15 SC); $RFC2_2$: Reduced fat cheese (0.015 XG and 0 SC); $RFC2_3$: Reduced fat cheese (0.03 XG and 0.45 SC); $RFC2_4$: Reduced fat cheese (0.045 XG and 0.3 SC); HFC_1: High fat cheese (0 XG and 0 SC); HFC_2: High fat cheese (0.015 XG and 0.15 SC); HFC_3: High fat cheese (0.030 XG and 0.30 SC); HFC_4: High fat cheese (0.045 XG and 0.45 SC)

Table 4. Analysis of variance for means base on Taguchi design

Independent variable	*F* ratio	*p* val
Fat	221.60	0.000*
Xanthan gum	0.68	0.593
Sodium caseinate	14.27	0.004*

* Significant ($p < 0.05$).

Table 5: The significant (p<0.05) of each independent variable effect base on full factorial design indicated by using *F*-ratio and *p* Val

Response variable		Independent variable		
		%Fat in cheese milk	Sodium caseinat (gr/100)	Fat *sodium caseinate
Asp	*p* Val	0.000*	0.001*	0.000*
	F ratio	506.43	10.13	17.87
Glu	*p* Val	0.000*	0.109*	0.000*
	F ratio	1194.03	2.37	22.31
Ser	*p* Val	0.000*	0.006*	0.000*
	F ratio	692.41	6.12	29.08
His	*p* Val	0.000*	0.001*	0.000*
	F ratio	366.77	10.04	17.12
Gly	*p* Val	0.000*	0.000*	0.000*
	F ratio	1938.08	13.66	47.20
Thr	*p* Val	0.000*	0.773	0.000*
	F ratio	1490.73	0.37	20.68
Arg	*p* Val	0.000*	0.001*	0.000*
	F ratio	696.45	9.33	35.37
Ala	*p* Val	0.000*	0.385	0.000*
	F ratio	311.34	1.08	7.57
Tyr	*p* Val	0.000*	0.012*	0.000*
	F ratio	611.56	5.08	15.59
Cys	*p* Val	0.000*	0.003*	0.000*
	F ratio	725.78	7.35	27.09
Val	*p* Val	0.000*	0.735	0.000*
	F ratio	1001.78	0.43	14.95
Met	*p* Val	0.000*	0.005*	0.000*
	F ratio	1242.85	6.18	30.17
Phe	*p* Val	0.000*	0.112	0.000*
	F ratio	922.43	2.34	21.39
Iso	*p* Val	0.000*	0.001*	0.000*
	F ratio	585.37	8.76	21.23
Leu	*p* Val	0.000*	0.033*	0.000*
	F ratio	1647.22	3.73	31.39
Lys	*p* Val	0.000*	0.063	0.000*
	F ratio	802.31	2.97	25.28
Pro	*p* Val	0.000*	0.004*	0.000*
	F ratio	1923.43	6.80	33.34

* Significant ($p < 0.05$).

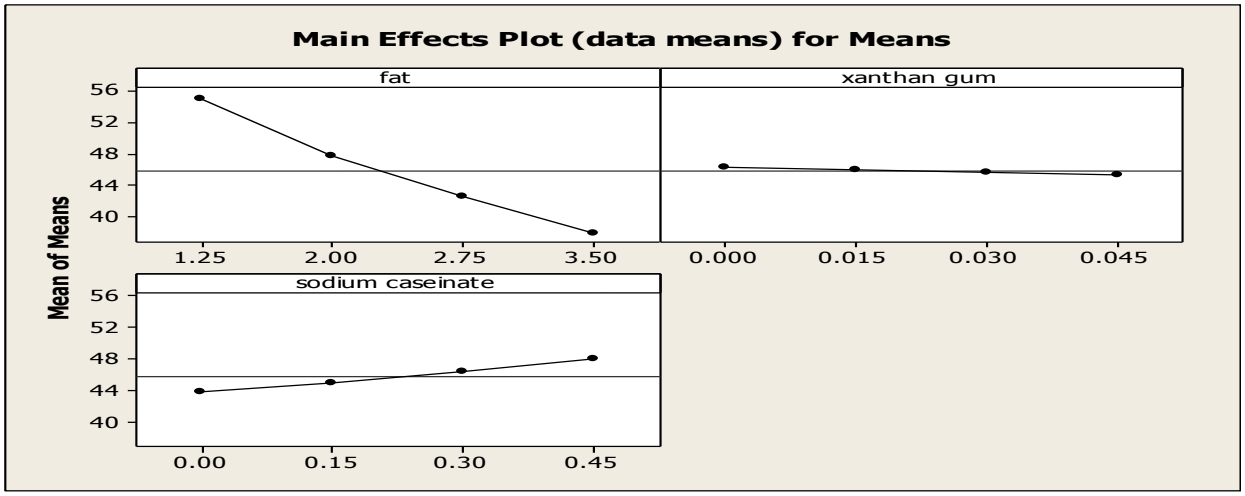

Figure1: main effect of mean base on Taguchi design