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Optimization of processing parameters for extraction of total, insoluble and soluble dietary fibers of defatted rice bran

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Abstract

Response surface methodology was used to optimize the processing parameters for extraction of total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) of defatted rice bran (DRB). The studied independent factors were concentration of NaOH solution (varying from 0.15 to 0.25 mol/L), soaking time (from 60 to 90 min), α-amylase enzyme – substrate (E: S) ratio (from 0.6:100 to 0.9:100 g: g of dry DRB) and alcalase enzyme concentration (from 3.5:100 to 4.7 g: g of dry DRB)) whereas; the dependent variables were extraction yield and purity of TDF, IDF and SDF. Therefore, the three- level four-factor Box-Behnken design was used to establish the optimum conditions and the generated regression quadratic polynomial models and adequacy of each dependent variable were significant ($p < 0.0001$) with regression coefficient R2 (> 0.90) and lack of-fit was not significant. Moreover, ANOVA showed that most of the linear, interaction and quadratic regression coefficient values were significant ($p < 0.05$). The optimum processing parameters observed for extraction of TDF, IDF and SDF with high yield and purity were: 0.15 mol/L NaOH solution concentration, 64.3 min soaking time, 0.68:100 and 3.52:100 (g: g) α-amylase and alcalase enzyme – substrate ratio (E: S), respectively. Moreover, the alkali pretreatment was the factor amongst the others that significantly ($p<0.05$) affected the purity of Fiber fractions but did not contribute to improve their yields.

Key words: Dietary fibers, Optimization, Purity, Response surface methodology, Yield

Introduction

The measurement of dietary fibers in foods is complexes issue not only the choice of analytical method, but also the definition of fiber. The gravimetric techniques, which were earliest and included crude fiber, acid detergent fiber, and neutral detergent fiber, grossly underestimate dietary fiber content and are being replaced by new and more accurate methods (Dreher, 1987). Therefore, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) from food or plant material are determined using AOAC enzymatic-gravimetric method (Prosky et al., 1988).

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Different conditions for extracting TDF, IDF and SDF from cereal bran were previously studied by Cartaňo and Juliano (1970), Theandor and Westerlund (1986), Aoe et al. (1993) and Thumthanarak (1996). They reported that the yield and the quality of dietary fibers are dependent of the relevant conditions used to extract the fibers. Numerous other studies have also shown that the extraction of non-starch polysaccharides was significantly affected by the extraction conditions (Wu et al., 2007; Yin and Dang, 2008; Liu et al., 2009; Li et al., 2009; Qiao et al., 2009; Zhao et al., 2009; Guo et al., 2010; Sun et al., 2010; Zhu et al., 2010).

It is evident that the optimum TDF, IDF and SDF yields cannot be estimated based on just the one-factor (single factor approach). But, the interaction between extraction conditions may be included in the determination of the yield and quality. A study is needed not only to determine the optimum extraction conditions to obtain a desirable yield or quality of TDF, IDF and SDF from defatted rice bran but is also required to understand

the degree of interactions between the extraction conditions such as concentration of enzymes, soaking time in the solvent (NaOH) and solvent concentration. A statistical optimization procedure such as response surface methodology (RSM) includes the interaction of the extraction factors into computation (Haaland, 1989).

RSM is widely applied in the food industry to determine the effects of multiple processing variables and their interaction on response variables (Vasquez and Martin, 1998; Senanayake and Shahidi, 2002; Tellez-Luis et al., 2003). Myers and Montogomery (2002) have expressed that RSM can be used to develop, improve, and optimize a process because the methodology includes both statistical and mathematical techniques. The RSM reduces the number of experimental trials and is considered to be less laborious and time consuming to optimize a process (Glovanni, 1983). The RSM can also be applied to an experimental design such as Box-Behnken (BBD) with three factors and three level variables to fit a second-order polynomial by least squares (Liu et al., 2009).

The objectives of this study were to a) determine the optimal composition of DRB and DRB fiber content b) determine effects of single factors such as NaOH concentration, soaking time in the NaOH solution, concentrations of α-amylase and alcalase to DRB on TDF, IDF and SDF yield and purity, and c) optimize the processing parameters such as NaOH concentration, soaking time, concentrations of α-amylase and alcalase on the TDF, IDF and SDF yield and purity using RSM with a three level and four independent variables Box-Behnken factorial design.

Materials and Methods

Proximate analysis of defatted rice bran

Moisture, protein, fat and ash contents of DRB were determined according to AACC approved methods 44-15A, 4-13, 30-25 and 08-17 respectively (AACC 2000). TDF, IDF and SDF contents of DRB were determined according to AACC method 32-07 using an enzymatic-gravimetric method with phosphate buffer (0.08 M, pH 6.0).

Extraction of TDF, IDF and SDF from defatted rice bran

TDF, IDF and SDF were extracted (Figure 1) by a modified procedure of Mirko (Mirko et al., 2003). DRB (10 g) was pretreated with NaOH solution. DRB was soaked with NaOH solution at different concentrations during different times at room temperature. After chemical pretreatment DRB was separated by centrifugation and washed with distilled water to neutral pH and then TDF, IDF, and SDF were extracted by enzymatic - gravimetric procedure of Mirko et al. (2003).

For enzymatic hydrolysis two (2) types of α-amylase: termamyl α-amylase 20.000 U/g and α-amylase 3000 U/g (Wuxi Enzyme Factory), and four (4) types of protease: neutrase 0.8L, alcalase 2.4 L (Novozymes Biological Engineering Beijing), flavourzyme 500 LAPU/g, protamex 1.5 AU/g (Wuxi Enzyme Factory) were used to select the best one. Termamyl α-amylase and Alcalase were selected according their degree of hydrolyze (DH) (Unpublished data). Enzymatic hydrolysis was completed by incubation with amyloglucosidase (100000 U/g Novozymes Biological Engineering).

Optimization of extraction of TDF, IDF, and SDF from DRB

The best combinations of the variable factors for the extraction of TDF, IDF, and SDF were determined using a three levels and four independent variables Box-Behnken factorial Design (BBD) (Sun et al., 2009). The independent extraction variables including concentration of NaOH solution (X_1), soaking time (X_2), and α-amylase – dry DRB ratio (X_3), and alcalase – dry DRB ratio (X_4) were used for this study.

The optimum range of X_1, X_2, X_3, and X_4 were determined based on the single factor experiment for extraction of TDF, IDF, and SDF. The variables (X_1, X_2, X_3, and X_4) were coded according to Eq. 1) for statistical calculation:

$$i = (Xi - Xo) / \Delta X \quad \text{(Eq.1)};$$

Where i was the coded value of the variable; Xi was the actual value of the independent variable Xo was the actual value of the independent variable at the center point, and ΔX was the step change value of the independent variable.

Table 1. Independent variable and levels used in the Response Surface Design.

Independents variables	Factor levels		
	-1	0	1
X_1: Concentration of NaOH (mol/L	0.15	0.2	0.25
X_2: Soaking time (min)	30	60	90
X_3: α-amylase –dry DRB (E:S) ratio (g:g)	0.6	0.75	0.9
X_4: alcalase –dry DRB (E:S) ratio (g:g)	3.5	4.1	4.7

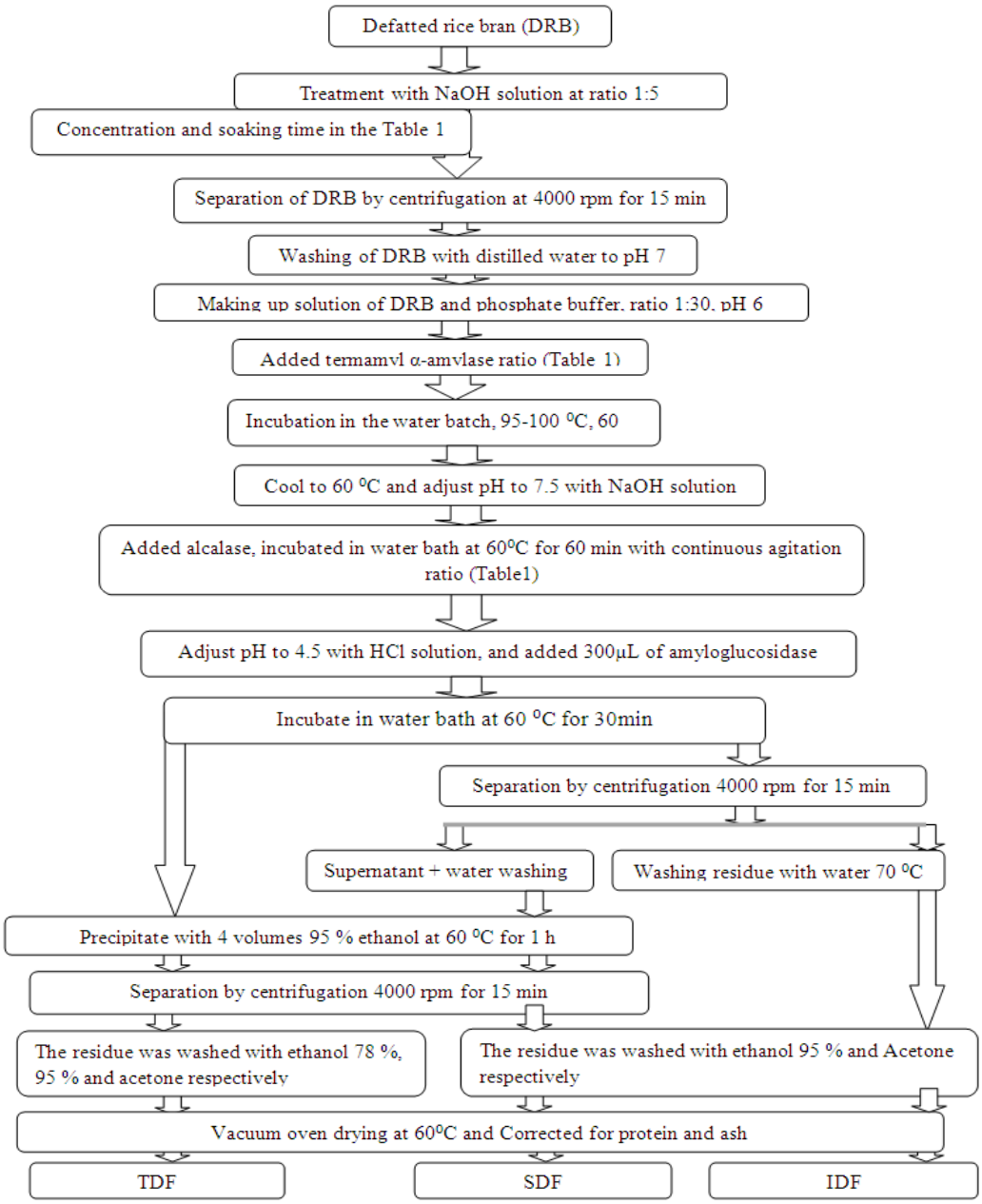

Figure 1. TDF, IDF, and SDF from DRB extraction flow diagram.

The dependents variables were the yields (%, w/w) and the purity (%) of TDF, IDF, and SDF from DRB in this experimental design. The complete experimental design consisted of 29 experimental points. All of the 29 treatments were taken in random order. The conditions at the center of BBD were repeated five times (2, 5, 6, 11, and 26) to estimate the pure error sum of squares (Zhu et al., 2010).

Data analysis

Mean values from the repeated or separated analyses were reported with standard deviation. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA).

Data from BBD were analyzed by multiple linear regressions to fit the quadratic polynomial model equation (2).

$$Y = \beta 0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \quad \text{(Eq. 2)}$$

Where, Y is the dependent variables (yield or purity of TDF, IDF, and SDF from DRB); β_0, β , β , and β are the coefficient regression of constant, linear, quadratic and interactive terms, respectively; and are the coded independent variables.

The regression coefficients of linear, quadratic and interaction terms were determined based on ANOVA and the P-value (0.05) of each coefficient was considered to be statistically significant. The 3-D response surface plots and contour plot were obtained using the response value along with two independent variables, while the other variables were the fixed constants at their respective 0 level (center value of the testing range).

Results and Discussion

Optimal composition of DRB

The Table 2 shows the proximate composition of defatted rice bran. The total dietary fiber (TDF) content of DRB is 32.98% (w/w) while, its IDF and SDF contents are 30.2% and 2.7% (w/w) respectively. These values of TDF and IDF contents were higher than those found by Abdul-Hamid while the values of SDF content were similar (Abdul-Hamid, 2000).These results showed that IDF represents the major component (93.84 %) while SDF content is only 6.16% (w/w) of rice bran dietary fiber.

Table 2. Chemical composition of defatted rice bran (DRB).

Proximate composition of DRB (% of dry weight of DRB)	
Moisture	8.7±0.03
Protein	16.2±0.2
Fat	2.8±0.05
Ash	10.7±0.01
TDF	32.9±0.3
IDF	30.2±0.4
SDF	2.7±0.15

Values are means ± standard deviation of 3 replicates (n=3) TDF =Total dietary fiber, IDF = Insoluble dietary fiber and SDF = Soluble dietary fiber

Effect of single factor condition on the yield and purity of TDF, IDF and SDF of DRB

The result of the extraction yield and the purity of TDF, IDF and SDF according to the Box-Behnken design matrix of four variables are shown in Table 3.The highest yield values were 31.11, 26.88 and 2.69 % under the extraction conditions 9, 13 and 7 for TDF, IDF and SDF, respectively. While, the highest purity values were 81.84, 90.00 and 53.57% under conditions 25 for TDF, IDF and 19 for SDF.

Effect of single factor on yield and purity of TDF

The yield of TDF ranged from 26.05 to 31.11%, while the purity ranged from 75.97 to 81.84%. The extraction yield increased with the decrease of NaOH concentration (Figure 2A) while increasing soaking time had moderate effect on it (Figure 2B). Jackson (1977) reported that alkali pretreatment is required to alter the structure of cellulosic biomass than dispirits the cell wall by dissolving hemicellulose and lignin, by hydrolyzing uronic and acetic acid esters and by swelling cellulose and decreasing the crystallinity of cellulose. Melinda et al. (2007) have confirmed in their study "Corn fiber as a raw material for hemicellulose and ethanol production". Thus, pretreatment with high concentration of alkali solution might cause some loss of polysaccharide which can explain the decrease of yield of TDF with increasing NaOH solution concentration.

Inversely the purity of TDF has been increased with the increase of NaOH concentration from 78.39 to 81.04% (Figure 2A). The purity of TDF have been increased during the first 60 min of soaking after that it is moderately decreased (Figure 2B). The alteration of the cell wall of lignocellulosic substances by alkali solution makes them more accessible to the enzymes (Melinda et al., 2007) which might improve the hydrolysis of starch and protein and then increased the purity of fiber.

The increase of Termamyl – DRB ratio from 0:100 to 0.75:100 increased the purity of TDF but, its yield was not significantly affected (Figure 2C). Alcalase - DRB ratio also affected the TDF purity. The yield was not affected with an increasing of alcalase – DRB ratio while, the purity was increased with an increase of E: S ratio from 3.5:100 to 4.1:100 and after that it decreased (Figure 2D).

Effect of single factor on yield and purity of IDF

Similar to TDF, the yield of IDF also increased at lower concentrations of NaOH (Figure 2A). But, the soaking time and enzymes: DRB ratio did not significantly affect the yield (Figure 2B, 2C and 2D). The purity of IDF was affected by the single factor in the same way as the purity of TDF (Figure 2A, 2B, 2C and 2D). The changes in IDF yield and purity due to the effects of single factors might have similar explanation with TDF.

Effect of single factor on yield and purity of SDF

The yield of SDF was moderately or not affected by all single factors (NaOH concentration, soaking time, and α-amylase and alcalase – DRB ratio) (Figure 2A, 2B, 2C and 2D). However, the purity of SDF increased with the increase of NaOH concentration and soaking time (Figure 2A and 2B). On other hand, the purity of SDF was higher when amylase – dry DRB ratio and alcalase – dry DRB ratio were 0.6:100 and 3.5:100 respectively. After that the purity decreased (Figure 2C and 2D).

Contrary to what has been reported by a number of authors (Cartaño and Juliano, 1970; Aoe et al., 1993; Yuting, 2008; Teramoto et al., 2008; Buranov and Mazza, 2010) that the chemical extraction (alkaline or acid) of SDF increases their yield, the alkaline pretreatment of DRB could not increase the yield of SDF. However, the pretreatment favorably improved their purity. The reasons are probably the same previously cited above for TDF and DTF.

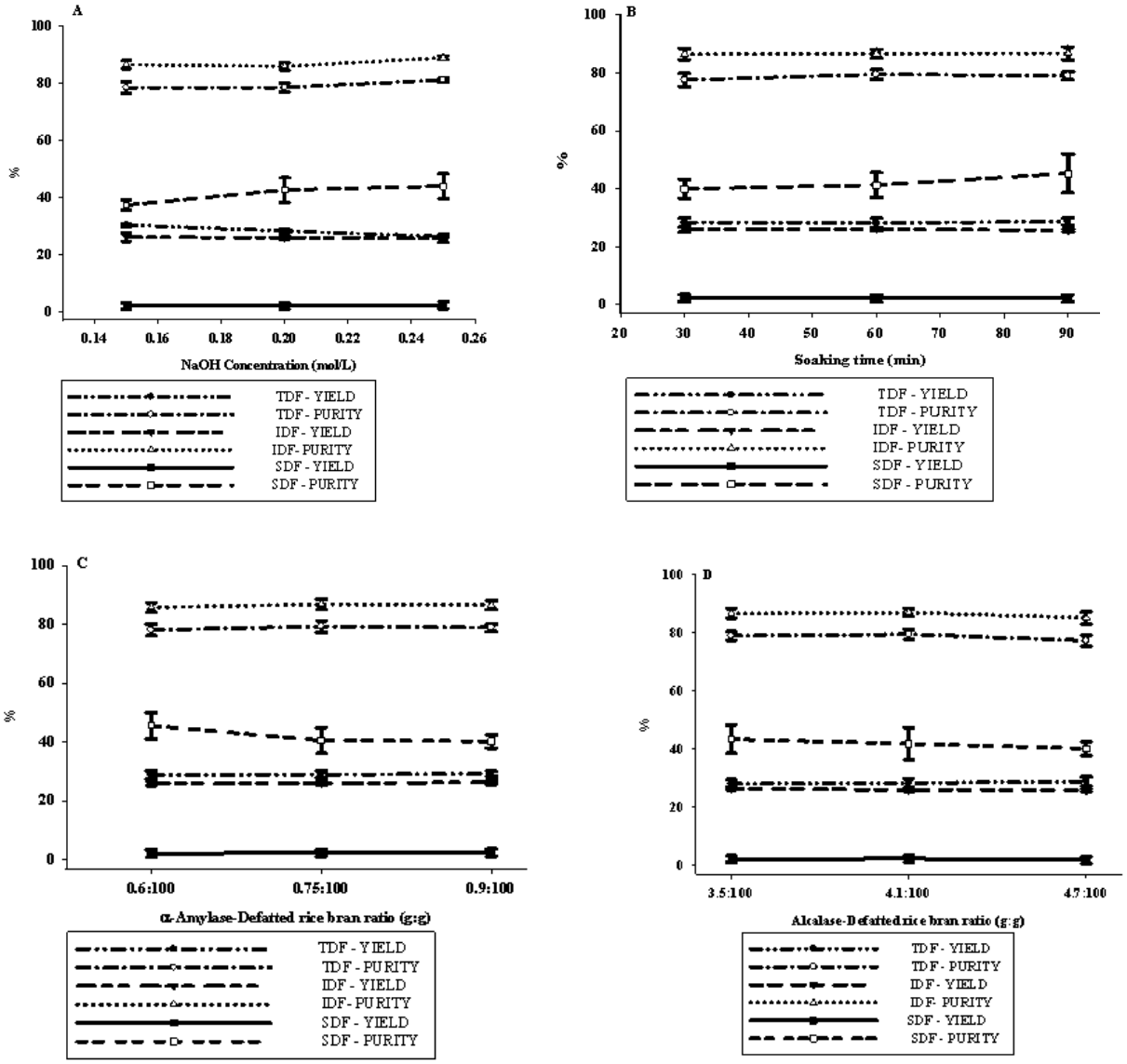

Figure 2. Effects of NaOH concentration (A) , soaking time in the NaOH solution (B), α-Amylase- Defatted rice bran ratio (C) and Alcalase- Defatted rice bran ratio (D) on the Yield and Purity of TDF, IDF and SDF from defatted rice bran.

Optimization of extraction conditions for TDF, IDF, and SDF from DRB

The extraction conditions were optimized by a second order polynomial equation using a 29-run BBD with four factors and three levels including five replicates at the center point. The experimental conditions according to the factorial design in coded units with the uncoded experimental values are shown in Table 3.

Optimization of extraction yield and purity of TDF

Maximum yield (Y1) and purity (Y2) of TDF were obtained under the extraction conditions of treatments 9 and 25-26 (Table 3), respectively. By analyzing the experimental data through multiple regressions, second-order polynomial equations were generated in terms of coded values as follow:

Yield: $Y1= 27.93 - 2.04X_1 + 0.14X_2 - 0.24X_3 + 0.39X_4 + 0.29X_1X_2 + 0.001256X_1X_3 – 0.30X_1X_4 – 0.36X_2X_3 – 0.12X_2X_4 + 1.22X_3X_4 + 0.20X_1^2 + 0.41X_2^2 + 0.22X_3^2 + 0.21X_4^2$

Purity: $Y2= 80.32 + 1.33X_1 + 0.73X_2 + 0.36X_3 - 0.81X_4 – 1.50\ X_1X_2 - 0.35X_1X_3 + 1.37X_1X_4 - 0.71X_2X_3 – 0.61\ X_2X_4 + 0.25X_3X_4 + 0.76\ X_1^2 - 1.46X_2^2 - 1.06X_3^2 - 1.58X_4^2$

The ANOVA of fitted quadratic polynomial model of extraction showed that the model was significant ($p<0.05$, n=3 and lack of fit was insignificant (Table 4).

Table 3. Box-Behnken Design Matrix of four variables.

Treatments	X_1	X_2	X_3	X_4	Yield TDF	Purity TDF	Yield IDF	Purity IDF	Yield SDF	Purity SDF
					Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_4 (%)	Y_5 (%)	Y_6 (%)
1	0	-1	0	-1	27.91	76.62	26.73	84.4	2.19	41.05
2	0	0	0	0	27.91	80.36	25.7	86.45	2.31	38.75
3	0	0	-1	1	27.75	76.23	25.46	83.88	1.66	38.45
4	1	0	0	-1	26.18	80.15	26.01	88.24	2.42	47.01
5	0	0	0	0	27.97	80.03	25.98	86.45	2.32	38.81
6	0	0	0	0	27.97	80.36	25.52	86.58	2.31	36.64
7	1	0	1	0	26.05	81.27	26.32	88.92	2.69	41.84
8	-1	-1	0	0	30.74	75.97	26.46	85.84	2.29	37.23
9	-1	0	0	1	31.11	76.04	26.16	84.34	1.94	37.91
10	-1	1	0	0	30.4	80.35	25.37	87.95	2.4	34.41
11	0	0	0	0	27.9	80.36	25.93	86.45	2.24	41.88
12	1	1	0	0	26.91	80.23	25.53	88.66	2.35	50.4
13	0	-1	1	0	28.54	78.34	26.88	85.58	2.14	44.34
14	0	0	-1	-1	29.41	78.23	26.58	85.29	2.07	49.38
15	-1	0	-1	0	30.65	78.18	26.51	85.7	2.16	39.47
16	0	1	1	0	28.13	78.15	25.03	87.1	2.43	44.36
17	0	0	1	1	29.74	77.59	26.59	84.55	2.03	43.49
18	1	0	0	1	26.4	81.14	25.93	88.52	2.23	38.89
19	0	1	-1	0	29.32	78.62	26.33	85	1.86	53.57
20	-1	0	0	-1	29.7	80.54	26.72	87.62	2.16	36.93
21	0	0	1	-1	26.53	78.61	26.57	86.24	2.19	39.49
22	0	-1	0	1	28.89	76.26	25.35	86.45	2.1	39.91
23	0	-1	-1	0	28.3	75.98	24.79	86.05	2.39	41.77
24	-1	0	1	0	30.12	79.23	25.95	87.05	2.26	38.23
25	1	-1	0	0	26.1	81.84	25.38	90	2.66	35.29
26	0	0	0	0	27.9	80.49	25.82	86.45	2.28	44.18
27	1	0	-1	0	26.57	81.61	25.39	88.54	2.15	50.56
28	0	1	0	-1	28.43	79.61	25.82	88.37	2.145	46.52
29	0	1	0	1	28.92	76.83	25.82	83.08	1.91	42.44

Table 4. ANOVA of fitted quadratic polynomial model of extraction Yield (Y1) and Purity (Y2) of TDF from DRB.

Source	DF		SS		MS		F value		Prob. > F	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Model*	14	14	61.31	97.42	4.38	6.96	3660.14	139.98	<0.0001	<0.0001
Residual	14	14	0.017	0.70	0.001197	0.050	—		—	
Lack of fit**	10	10	0.011	0.58	0.001126	0.058	0.82	1.96	0.6381	0.2695
Pure Error	4	4	0.005494	0.12	0.001373	0.029	—		—	
Total	28	28	61.33	98.12	—		—		—	

DF=Degree of Freedom; SS= Sum of Squares; MS= Mean Square, Model*= Model significant, and Lack of fit**= lack of fit not significant.

The analysis of variance, goodness-of-fit and the adequacy of the models showed that the models were found to be adequate for prediction with the range of selected experimental variables. The means were 28.36±0.035 and 78.94±0.22 for yield and purity, respectively. The determination coefficients (R^2= 0.9997 for yield and R^2= 0.9988 for purity) obtained by ANOVA of the quadratic regression models indicated that only 0.03 % and 0.02 % of the total variation were not explained by the models for yield and purity, respectively. The value of adjusted determination coefficients (Adjusted R^2 = 0.9995 and Adjusted R^2 = 0.9858 for yield and purity, respectively) indicated a high degree of correlation between the observed values, while a lower coefficient of variation values of yield (0.12) and purity (0.28) showed the experimental values were reliable (Liyana-Pathirana and Shahidi, 2005).

The regression parameters of the predicted response surface quadratic models for yield and purity of TDF from DRB was shown in Table 4 The results indicated that only the interaction between NaOH concentration and α-amylase –DRB ratio (X_1X_3) was not significant ($p>0.05$, n=3) for the extraction yield, while all linear, quadratic and interaction between factors were significant ($p<0.05$, n=3) for the purity of TDF. The P-value was used to verify the significance of each coefficient and to describe the degree of interactions strength between the variables (Muralidhar et al., 2001). A smaller P-value means the corresponding coefficient is more significant (Muralidhar et al., 2001).

The result of statistical analysis showed that the entire factor (NaOH concentration, soaking time, amylase and alcalase DRB ratio) significantly ($P<0.05$, n=3 for both models) affected the yield and purity of TDF (Table 5).

The interaction regression coefficients X_1X_3 of yield quadratic polynomial model was not significant, which indicated the interaction between NaOH solution and α-amylase – DRB ratio did not affected the yield of TDF. While, all the interaction regression coefficients of purity polynomial model were significant thus, the interactions between all independent factors (NaOH concentration, soaking time α-amylase and alcalase DRB ratio affected the purity of TDF.

Table 5. Regressions Coefficients of the Predicted Quadratic Polynomial Models.

Parameters	Coefficient Estimate		Standard Error		P-value*	
	Yield	Purity	Yield	Purity	Yield	Purity
X_1	-2.04	1.33	0.009986	0.18	< 0.0001	< 0.0001
X_2	0.14	0.73	0.009986	0.18	< 0.0001	< 0.0001
X_3	-0.24	0.36	0.009986	0.18	< 0.0001	< 0.0001
X_4	0.39	-0.81	0.009986	0.18	< 0.0001	< 0.0001
X_1X_2	0.29	-1.50	0.017	0.31	< 0.0001	< 0.0001
X_1X_3	0.001256	-0.35	0.017	0.31	0.0943	0.0076
X_1X_4	-0.30	1.37	0.017	0.31	< 0.0001	< 0.0001
X_2X_3	-0.36	-0.71	0.017	0.31	< 0.0001	< 0.0001
X_2X_4	-0.12	-0.61	0.017	0.31	< 0.0001	< 0.0001
X_3X_4	1.22	0.25	0.017	0.31	< 0.0001	0.0001

*P-value less than 0.0500 indicate model terms are significant (n=3)

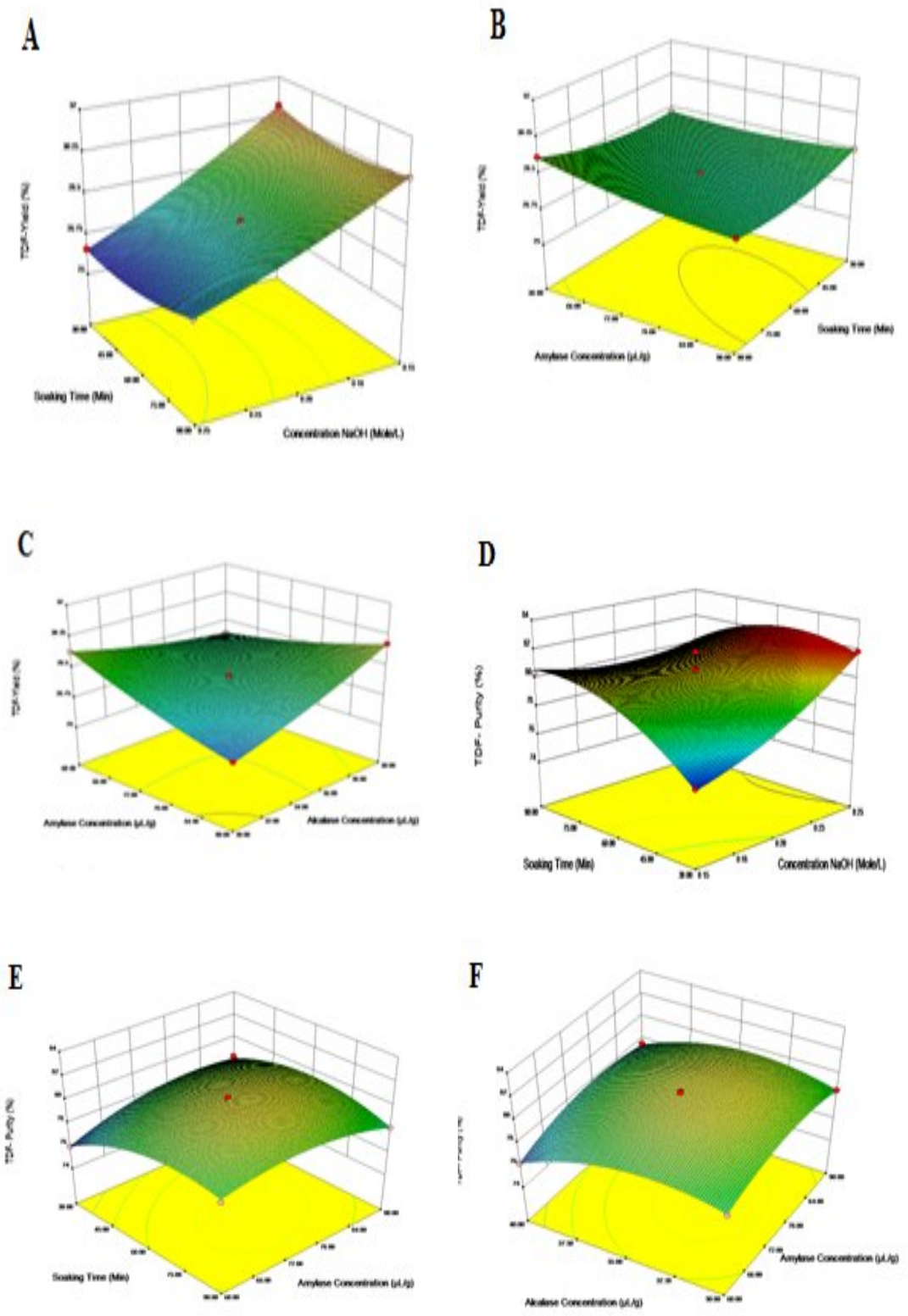

Figure 3. Response Surface Plots (3-D) showing the effects of interaction of some variable factors on yield and purity of TDF from defatted rice bran.

Table 6. ANOVA of fitted quadratic polynomial model of extraction Yield (Y3) and Purity (Y4) of IDF from DRB.

Source	DF		SS		MS		F value		Prob. > F	
	Y_3	Y_4	Y_3	Y_4	Y_3	Y_4	Y_3	Y_4	Y_3	Y_4
Model*	14	14	8.07	78.82	0.58	5.63	24.06	2074.73	<0.0001	<0.0001
Residual	14	14	0.34	0.038	0.024	0.002714	—		—	
Lack of fit**	10	10	0.20	0.024	0020	0.002447	0.59	0.81	0.7725	0.6456
Pure Error	4	4	0.14	0.014	0.034	0.003380	—		—	
Total	28	28	8.40	78.86	—		—		—	

DF=Degree of Freedom; SS= Sum of Squares; MS= Mean Square, Model*= Model significant, and Lack of fit**= lack of fit not significant

The response surface plots (3-D) of yield and purity of TDF from DRB affected by the interaction of NaOH concentration and soaking time, the interaction of soaking time and α-amylase concentration and the interaction between ratios of α-amylase-DRB and alcalase- DRB are shown in the Figure 3.

We fond that an increasing of the NaOH solution concentration and soaking time has led in lower yield and higher purity of TDF (Figure 3 A and D). However the combination of a short soaking time with a low DRB-α-amylase ratio gives a high yield of TDF (Figure 3 B) whereas, the combination of low α-amylase- DRB with alcalase- DRB ratio also leads to a high yield (Figure 3 C). The higher purity was reached when the soaking time and α-amylase-DRB ratio or ratios of α- amylase and alcalase were in the range (Figure 3 E, F).

As mentioned above alkali altered cell wall of lignocellulosic substance (Jackson, 1977) thus, the long soaking time of DRB in the high concentrate NaOH solution might cause the loss of some soluble polysaccharide hence the low yield of TDF. While, the short soaking time of DRB will favorite the access of enzyme to substrate which can improve the yield. Another hand the higher purity was due to the alkali pretreatment which facilitates the access of enzymes to DRB matrix therefore improves the degree of hydrolysis starch and protein.

Optimization of extraction yield and purity of IDF

Maximum yield (Y_3) and purity (Y_4) of IDF were obtained under the extraction conditions of treatments 13 and 25 (Table 3), respectively. The second-order polynomial equations were generated in terms of coded values as follow:

Yield: $Y_3 = 25.79 - 0.22X_1 - 0.14\ X_2 + 0.19X_3 - 0.26X_4 + 0.31X_1X_2 + 0.37X_1X_3 + 0.12X_1X_4 - 0.85\ X_2X_3 + 0.34\ X_2X_4 + 0.28X_3X_4 + 0.084X_1^2 - 0.20X_2^2 + 0.17X_3^2 + 0.34X_4^2$

Purity: $Y4 = 86.48 + 1.20X_1 + 0.15X_2 + 0.42X_3 - 0.78X_4 - 0.86X_1X_2 - 0.24X_1X_3 + 0.89X_1X_4 + 0.64X_2X_3 - 1.84X_2X_4 - 0.070X_3X_4 + 1.63X_1^2 + 0.016X_2^2 - 0.56X_3^2 - 0.92X_4^2$

The ANOVA of fitted quadratic polynomial model of extraction showed that the model was significant ($p<0.05$, n=3) and lack of fit insignificant (Table 6).

The analysis of variance, goodness-of-fit and the adequacy of the models showed that the R-square values indicated that only 3.99% (R^2 = 96.01%) and 0.05 % (R^2 = 99.95%) of the total variation were not explained by the models of extraction yield and purity of IDF, respectively. Similar to TDF the adjusted R-square (92.02% and 99.90% for yield and purity, respectively) also indicated a high degree of correlation between the observed values, while a lower coefficient of variation values of yield (0.60) and purity (0.060) showed the experimental values were reliable (Liyana-Pathirana and Shahidi, 2005).

The regression parameters of the predicted response surface quadratic models for yield and purity of IDF from DRB was shown in Table 7. The results indicated that X_1X_4 and X_1^2 were not highly significant ($p>0.05$ n=3) for extraction yield, while X_2^2 was not significant ($p>0.05$, n=3) for purity of IDF.

According ANOVA the yield and purity of IDF were significantly ($p<0.05$ n=3) affected by all the variables factors except interaction between concentrations of NaOH and alcalase and quadratic NaOH solution concentration ($P>0.05$ n=3).

The response surface plots (3-D) of yield and purity of IDF from DRB affected by the interaction of NaOH concentration and soaking time, the interaction of soaking time and α-amylase concentration and the interaction between ratios of α-amylase-DRB and alcalase-DRB are shown in the Figure 4.

The effects of interactions between NaOH concentration-Soaking time, α-amylase-DRB ratio-Soaking time and α-amylase-DRB ratio-alcalase-DRB ratio on the yield and purity of IDF were similar to TDF.

Table 7. Regressions Coefficients of the Predicted Quadratic Polynomial Models.

Parameters	Coefficient Estimate		Standard Error		P-value	
	Yield	Purity	Yield	Purity	Yield	Purity
X_1	-0.22	1.20	0.045	0.015	0.0003	< 0.0001
X_2	-0.14	0.15	0.045	0.015	0.0073	< 0.0001
X_3	0.19	0.42	0.045	0.015	0.0008	< 0.0001
X_4	-0.26	-0.78	0.045	0.015	< 0.0001	< 0.0001
X_1X_2	0.31	-0.86	0.077	0.026	0.0013	< 0.0001
X_1X_3	0.37	-0.24	0.077	0.026	0.0003	< 0.0001
X_1X_4	0.12	0.89	0.077	0.026	0.1384	< 0.0001
X_2X_3	-0.85	0.64	0.077	0.026	< 0.0001	< 0.0001
X_2X_4	0.34	-1.84	0.077	0.026	0.0005	< 0.0001
X_3X_4	0.28	-0.070	0.077	0.026	0.0025	0.0177

P-value less than 0.0500 indicate model terms are significant (n=3)

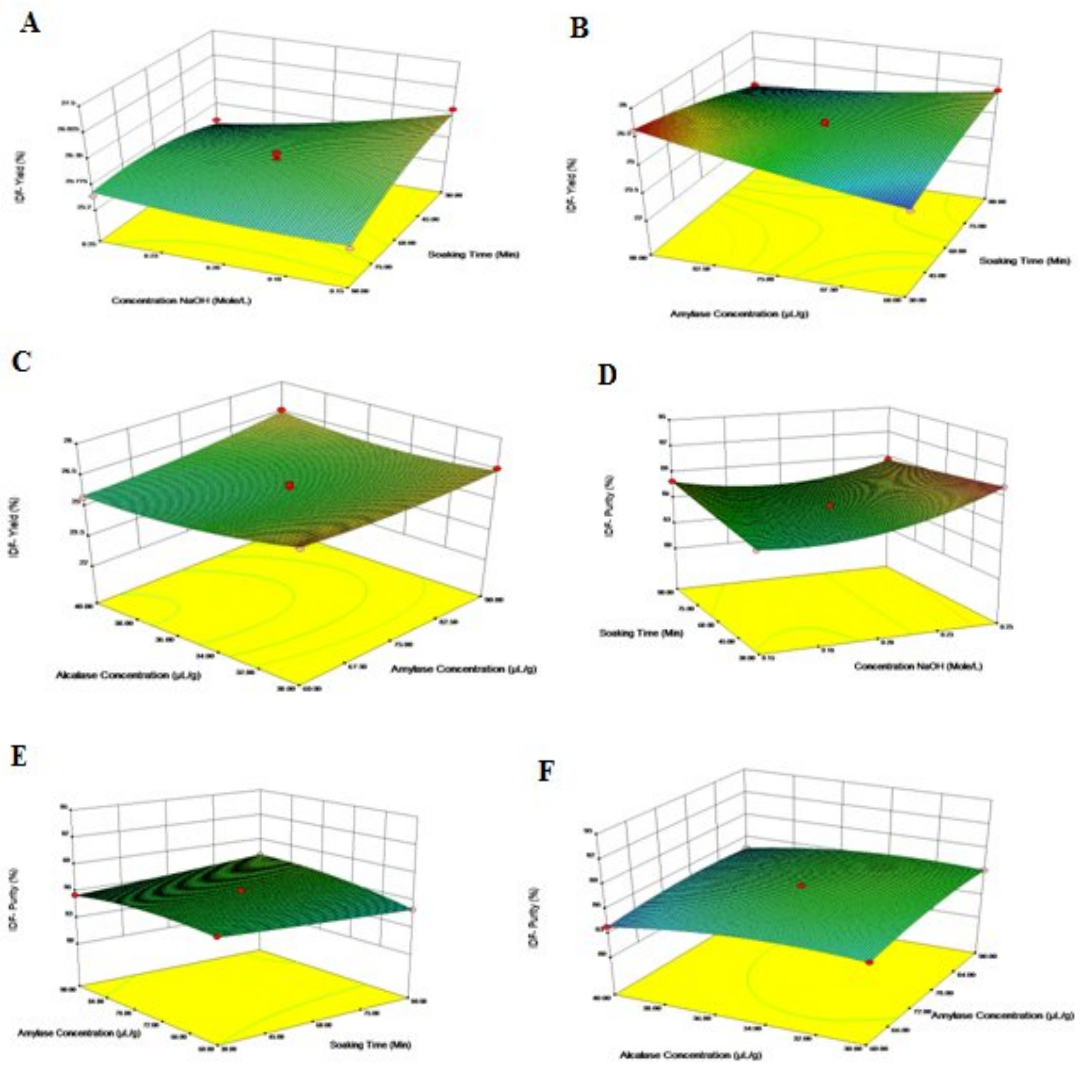

Figure 4. Response Surface plots (3-D) showing effects of interaction of some variable factors on the yield and purity of IDF from defatted rice bran.

Table 8. Analysis of variance of fitted quadratic polynomial model of extraction yield (Y5) and purity (Y6) of SDF from defatted rice bran.

Source	DF		SS		MS		F value		Prob. > F	
	Y_5	Y_6	Y_5	Y_6	Y_5	Y_6	Y_5	Y_6	Y_5	Y_6
Model*	14	14	1.29	604.50	0.092	43.18	41.06	9.72	<0.0001	<0.0001
Residual	14	14	0.031	62.16	0.0022	4.44	—		—	
Lack of fit**	10	10	0.027	26.92	0.0027	2.69	2.73	0.31	0.1730	0.9416
Pure Error	4	4	0.004	35.25	0.0010	8.81	—		—	
Total	28	28	1.32	666.67	—		—		—	

DF=Degree of Freedom; SS= Sum of Squares; MS= Mean Square, Model*= Model significant, and Lack of fit**= lack of fit not significant

Table 9. Regressions Coefficients of the Predicted Quadratic Polynomial Models.

Parameters	Coefficient Estimate		Standard Error		P-value	
	Yield	Purity	Yield	Purity	Yield	Purity
X_1	0.11	3.32	0.014	0.61	< 0.0001	< 0.0001
X_2	-0.056	2.67	0.014	0.61	0.0012	0.0006
X_3	0.12	-1.79	0.014	0.61	< 0.0001	0.0108
X_4	-0.11	-1.61	0.014	0.61	< 0.0001	0.0193
X_1X_2	-0.11	4.48	0.024	1.05	0.0005	0.0008
X_1X_3	0.11	-1.87	0.024	1.05	0.0004	0.0979
X_1X_4	0.008128	-2.28	0.024	1.05	0.7367	0.0486
X_2X_3	0.20	-2.94	0.024	1.05	< 0.0001	0.0143
X_2X_4	-0.037	-0.74	0.024	1.05	0.1369	0.4965
X_3X_4	0.062	3.73	0.024	1.05	0.0195	0.0033

P-value less than 0.0500 indicate model terms are significant (n=3)

Optimization of extraction yield and purity of SDF

Maximum yield (Y_5) and purity (Y_6) of SDF were obtained under the extraction conditions of treatments 7 and 19 (Table 3), respectively. The second-order polynomial equations were generated in terms of coded values as follow:

Yield: $Y_5 = 2.29 + 0.11X_1 - 0.056X_2 + 0.12X_3 - 0.11X_4 - 0.11X_1X_2 + 0.11X1X3 + 0.008128X_1X_4 + 0.20X_2X_3 - 0.037X_2X_4 + 0.062X_3X_4 + 0.12X_1^2 + 0.011X_2^2 - 0.094X_3^2 - 0.22X_4^2$

Purity: $Y_6 = 40.05 + 3.32X_1 + 2.67X_2 - 1.79X_3 - 1.61X_4 + 4.48X_1X_2 - 1.87X_1X_3 - 2.28X_1X_4 - 2.94X_2X_3 - 0.74X_2X_4 + 3.73X_3X_4 - 1.21X_1^2 + 1.68X_2^2 + 3.39X3^2 + 0.45X_4^2$

The ANOVA of fitted quadratic polynomial model of extraction showed that the model was significant ($p<0.05$ n=3) and lack of fit insignificant (Table 8).

The analysis of variance, goodness-of-fit and the adequacy of the models showed the R-square values indicated that only 2.38% and 9.32% of the total variation were not explained by the models of extraction yield and purity of SDF, respectively while, the adjusted R-square 95.25% and 81.35% also indicated high degree of correlation between values. The regression parameters of the predicted response surface quadratic models for yield and purity of SDF from DRB was shown in Table 9.

The results indicated that X_1X_4, X_2X_4 and X_1^2 were not highly significant ($p>0.05$ n=3) for extraction yield, while X_1X_3, X_2X_4; X_1^2; X_2^2 and X_4^2 were not significant ($p>0.05$ n=3) for purity of SDF. Thus, the statistical analysis showed that the yield of SDF was significantly ($P < 0.05$) affected by the independent variable factors. Only the interactions between alcalase-NaOH concentration and alcalase-soaking time in NaOH solution showed no significant effect on the extraction the yield of SDF. However, the P-value of NaOH, amylase, alcalase and interaction between enzyme ratios were the smallest ($P < 0.0001$) which indicated that these factors highly affected the extraction of SDF.

The purity of SDF was affected by all single factor (all exhibited small P-value) and the P-value of NaOH concentration were the smallest (<0.0001). Therefore, NaOH concentration had more effect on the purity of SDF compared to other factors. Only the interactions of NaOH-amylase-DRB ratio, soaking time-alcalase-DRB ratio, quadratic NaOH concentration, soaking time and alcalase-DRB ratio did not significantly ($P > 0.05$ n=3) affected the purity of SDF. However; the purity was more affected by the interactions between the concentration and soaking time of NaOH solution and between enzyme concentrations (smallest P-value).

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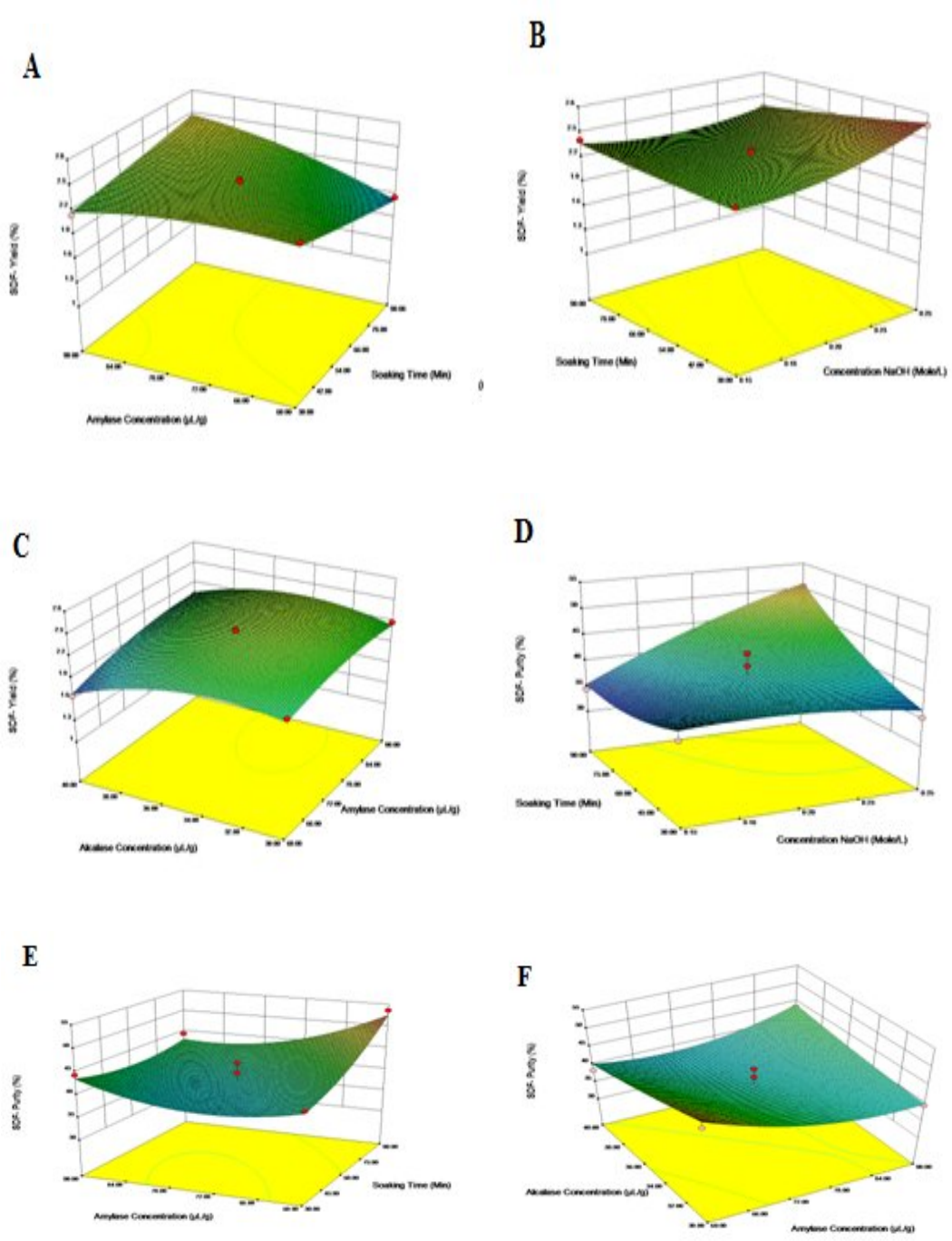

Figure 5. Response Surface Plots (3-D) showing effects of interaction of some variable factors on the yield and purity of SDF from defatted rice bran.

The response surface plots (3-D) of yield and purity of SDF from DRB affected by interaction of NaOH concentration and soaking time, the interaction of soaking time and α-amylase concentration and the interaction between ratios of α-amylase and alcalase to DRB are shown in the Figure 5.

The interaction between α-amylase-DRB ratio and soaking time showed that higher yield of SDF was reached when the ratio and time were increased

while, longer soaking time combined with ratio in range improved the purity of SDF (Figure 5 A and E). However, the combination of a short soaking time with a low NaOH concentration gives a high yield of SDF at the same time the increasing of these parameters increased its purity (Figure 5 B and D). On the other hand the combination of the alcalase- DRB ratio in the range with increasing α-amylase- DRB ratio also leads to a high yield of SDF while, its purity increased with increasing of these parameters (Figure 5 C and F).

Based on this optimization, the optimum extraction conditions under which the yields and purities of IDF, SDF and TDF of DRB could be obtained with desirability of 73% were as follow: the concentration of NaOH 0.15 mol/L, the soaking time in the NaOH solution 64.03 min, the α-amylase – DRB ratio 0.68:100, and the alcalase – DRB ratio 3.52:100, respectively.

The optimum yields and purities of IDF SDF and TDF were:

IDF: yield: 27.44%, purity: 86.99%

SDF: yield: 2.35%, purity: 51.57%

TDF: yield 31.50%, purity: 79.71%

The prediction factor levels for optimal extraction of IDF, SDF and TDF were the following concentration of NaOH solution 0.15, soaking time in the NaOH solution 64.03 min, α-amylase-DRB ratio 0.68:100 and alcalase-DRB ratio 3.52:100.

The prediction yield and purity of IDF, SDF and TDF were: IDF-Yield 27.44%, IDF-Purity 86.99%, SDF-Yield 2.35%, SDF-Purity 51.57%, TDF-Yield 31.50% and TDF-Purity 79.71%.

The optimal levels of the factors of the extraction of IDF, SDF and SDF from DRB and the prediction responses (yield and purity) were confirmed with 95% confidence of these fiber fractions.

Conclusion

ANOVA showed most of the independent variable factors and their combinations significantly ($p<0.05$) affected the yield and purity of all three fiber fractions of DRB. The yield and purity of TDF and the purity of IDF were affected at the same level by all variable factors ($P < 0.0001$). However, the yield of SDF was more affected by NaOH concentration ($P < 0.0001$) while, its purity was less affected by soaking time ($P = 0.0012$) compared to other factors.

Highly correlated second-order polynomial model used to optimize the extraction yield of IDF, SDF and TDF from DRB showed that:

- The interactions between all variable factors except that between concentration of NaOH and amylase concentration, significantly affected the extraction yield of TDF,
- Only the interaction between concentrations of NaOH and alcalase has no significant effect on the yield of IDF,
- The yield of SDF was affected by the interactions between all single factors except that between NaOH–alcalase concentration and soaking time–alcalase concentration.

For the purity of IDF, SDF and TDF the polynomial model showed that:

- The purity of IDF and TDF has been affected by the interactions between all variable factors. While, the SDF purity was also affected by the interactions between these all factors except interactions between NaOH-amylase concentrations and soaking time-alcalase concentration.

The optimization study demonstrated that:

- The optimum extraction yield of TDF, IDF and SDF were 31.11, 26.88 and 2.69%, respectively
- The optimum purity of TDF, IDF and SDF were 81.84, 90, and 53.57%, respectively.

The optimum levels of independent extraction variables were the following: concentration of NaOH 0.15 mol/L, soaking time 64.03 min, α-amylase – DRB ratio 0.68:100, and the alcalase – DRB ratio 3.52:100.

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FOOD SCIENCE AND NUTRITION

Effects of sugar type and concentration on the characteristics of fermented turi (*Sesbania grandiflora* (L.) Poir) milk

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Abstract

Fermented milk products are widely consumed for their positive health image, which can be further enhanced by the addition of probiotic bacteria with therapeutic properties. One of the fermented milk products is fermented turi-milk; however, the turi milk has unpleasant off flavor and sour taste. The objective of this study was to determine the best treatment in producing fermented turi-milks with acceptable flavor and taste. Two treatments were arranged in Randomized Block Design with 3 replications. The first treatment was sugar types which consisted of sucrose, glucose, and a sucrose-glucose mixture at a ratio of 1:1. The second treatment was concentrations of sugar consisting of 29.25%, 35.75%, 42.25%, and 48.75% (w/v). Clean turi seeds were submerged in 0.5% NaHCO3 solution at 80oC for 15 minutes, crushed using a blender, and filtered with filter cloth to obtain turi milk. The turi milk was added with 10% skim milk powder and 4% glucose, inoculated with *Lactobacillus casei* subsp. rhamnosus, and then incubated at 37oC for 96 hours to produce fermented turi-milks. The fermented turi-milks were added with sugar at different concentrations and then stored at refrigerate temperature for 12 days. After storing for 12 days, samples of the turi-milks were taken, and analyzed to determine their pH, total acid bacteria, and sensory characteristics. Result of this study showed that adding sucrose at a concentration of 35.75% (w/v) was the best treatment to produce fermented turi-milk with acceptable flavor and taste. The best fermented turi-milk had a pH of 3.71, a total lactic acid bacteria of 3.62 x 1011 cfu/g, a taste scores of 3.75, a flavor score of 3.3, and an overall acceptance score of 3.75 (out of 5 scales).

Key words: Fermentation, Fermented milk, Turi, *Sesbania grandiflora*, Sugar

Introduction

Fermented milk products are widely consumed for their benefits and refreshing effects. Their popularity is said to be attributed to the effective use of consumer-driven flavors and milder cultures (Jensen and Kroger, 2000). Valli and Traill (2005) stated that these products already have a positive health image, which can be further enhanced by the addition of probiotic bacteria with therapeutic properties (Lourens-Hattingh and Viljoen, 2001).

Probiotics are living microorganisms that when consumed in sufficient amounts provide health benefits beyond basic nutrition (Food and Agricultural Organization of the United Nation/World Health Organization, 2002). Potential benefits of these probiotic microorganisms in health conditions are primarily in prevention of common infectious diseases, such as diarrhea, necrotizing enterocolitis, and allergies (Vanderhoof et al., 1999; Rosenfeldt et al., 2002; Mastrandrea et al., 2004; Weizman et al., 2005; Lin et al., 2005, 2009).

Because of health benefits, the probiotic bacteria are emerging as important dietary ingredients in functional foods. Majorities of the probiotics are lactic acid bacteria, especially lactobacilli, and bifidobacteria (Schrezeomeir, 2008). Factors related to technological and sensory aspects of the probiotic food products are of utmost importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional products in the future (Mattila-Sandholm et al., 2002).

One of the fermented milk products is fermented turi-milk. Turi (*Sesbania grandiflora* L. Poir) - a plan belong to Leguminosae - grows on tropical and subtropical areas and yields turi beans

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consisting of 38.4% protein, 44.7% carbohydrate, 4.3% fat, and 12.6% water (Anonymous, 2010a). Because of its high protein and carbohydrate contents, turi-beans are utilized as raw material of turi-milk, which is not technically milk, but a beverage made from turi beans (Nurhayati et al., 1996). However, the turi-milk has unpleasant off flavor because of lipoxidase-catalyzed oxidation of unsaturated turi-bean oil. To eliminate the problem, Indriyani (2002) fermented the turi milk using lactic acid bacteria as a starter to produce fermented turi-milk. Flavor of the fermented turi-milk was acceptable, but its taste was too sour because of lactic acid content (Irawan, 2003).

In order to reduce sour taste of fermented turi-milks, sugar was added to the fermented turi-milks. If sugar addition is too much, the fermented turi-milk will be too sweet and lactic acid bacteria cannot survive; if too small, the sour taste of the turi-milk still exists. Thus, objective of this study were to find out the treatment (adding sugar) in producing fermented turi-milks with acceptable flavor and taste.

Materials and Methods

Materials

Materials used in this study consisted of turi seed obtained from South Lampung, Indonesia, *Lactobacillus casei* subsp. *rhamnosus* obtained from Biotechnology Laboratory, Institut Pertanian Bogor, Indonesia, and glucose, sucrose, skim milk, $NaHCO_3$, aquadest, MRSA media, MRSB media, as well as NaCl which obtained from the Deparatment of Agricultural Product Technology, the University of Lampung, Indonesia.

Experimental design

Two treatments in this study were arranged in Randomized Block Designed with 3 replications. The first treatment was the types of sugar which consisted of sucrose, glucose, and sucrose-glucose mixture at a ratio of 1:1. The second treatment was the concentration of sugar added into fermented turi-milk and consisted of 29.25%, 35.75%, 42.25%, and 48.75% (w/v). The fermented turi-milks were analyzed to determine their total lactic acid bacteria, pH, and sensory characteristics (taste, flavor, and overall acceptance). Data of total lactic acid bacteria and pH were analyzed using analysis of variances and performed Barlettt and Turkey tests to differentiate among treatments (Steel and Torrie, 1995). Polinomial orthogonal test was also carried out to determine effect tendencies of the treatments. Data of sensory characteristics were presented in bar diagram and discussed descriptively.

Experimental Procedure

Production of turi milk

Turi milk was produced according to a method developed by Nurhayati et al. (1996). After separating from dirt and damaged seed, turi seed was washed upto clean, and then submerged in 0.5% (w/v) $NaHCO_3$ solution at 80^{o}C for 12 hours. A ratio of the $NaHCO_3$ solution and turi seed weight was 3 to 1 (v/w). Furthermore, the turi seed was drained, and then blanced in a solution of 0.003% (w/v) $NaHCO_3$ for 15 minutes. After peeling, the turi seed was crushed using a blender and added hot (80ºC) water with a ratio of 1 to 6. The result of the destruction in the form of gruel was filtered with filter cloth to obtain filtrate, namely turi milk.

Starter preparation

Pure culture *of Lactobacillus casei* subsp. *rhamnosus* in ampoules was entirely transferred to the erlenmeyer containing 50 mL of MRS Broth medium and then incubated for 48 hours at a temperature of 37^{o}C. Two drops of the incubated MRS Broth medium were inoculated into 50 mL of turi milk which has been added with 10% (w/v) skim milk and sterilized at a temperature of 121ºC for 15 minutes. After incubation at 37ºC for 48 hours, the culture was used as parent culture. The parent culture was then inoculated into the same medium as much as 0.5% (v/v) and then incubated for 48 hours at a temperature of 37ºC. After incubation, the result culture was used as between culture. As much as 0.5% (v /v) of the between culture was inoculated into the same medium with the addition of 3.0% glucose. The culture was incubated at a temperature of 37ºC for 24 hours in order to obtain working culture which was ready to be used.

Production of fermented turi milk

Fermented turi-milk was produced according to the method developed by Indriyani (2002). Turi milk was added with 10% (w/v) skim milk powder and 4% (w/v) glucose. The mixture was mixed homogenoiusely, heated at 80ºC for 30 minutes, and then cooled upto 37ºC. After cooling, the mixture was inoculated with working culture and then incubated at a temperature of 37 ºC for 96 hours to produce fermented turi-milk. The fermented turi-milk was added with different types of sugar (sucrose, glucose, or sucrose and glucose mixture at a ratio of 1:1) at concentrations of 29.25%, 35.75%, 42.25%, and 48.75% (w/v) and then stored at refrigerate (5 – 10^{o}C) temperature for 12 days.

Production of sugar solution

Sugar solution was produced by pouring 325 g sugar into 500 mL measuring flask, adding with

water up to tera sign, and shaking until all sugar was dissolved. The sugar solution containing 65% sugar (w/v) was then pasteurized at a temperature of 65ºC for 30 minutes. This solution was then added into fermented turi-milks up to sugar concentration treatments were achieved.

Experiment observation

Experiment observations were performed to determine pH value (AOAC, 1990), total lactic acid bacteria (Fardiaz, 1987), and sensory characteristics (taste, flavor, and overall acceptance) (Soekarto, 1985). Observations of total lactic acid bacteria were conducted every four days during the 12 days storage of fermented turi-milk in order to determine the effect of sugar on the growth of lactic acid bacteria. Sensory test and pH analysis were performed after 12 days storage of the fermented turi-milks at refrigerate temperature.

Results and Discussion

pH value

After collecting and tabulating pH data, analysis of variance was carried out. Results of the analysis showed that the type of sugar had a very significant effect on the pH of the fermented turi-milks but the the concentration of sugar and interaction between the two treatments had no significant effects. pH values and their linear lines were presented at Figure 1. pH values of fermented turi-milks added with glucose were lower (3.64 – 3.67) than that added with sucrose (3.71 – 3.75). It indicates that the lactic acid microba (*L. casei subsp rhamnosusin*) still alive and apparently preferred to utilize glucose than sucrose for producing lactic acid. This finding is similar to the finding of Wang et al. (1974), who also found that fermented soymilk with *L. acidophilus B-1911* had a lower pH when added with glucose than that added with sucrose.

Total of lactic acid bacteria

Total lactic acid bacteria were determined after storing the fermented turi-milks for 0, 4, 8, and 12 days. After collecting and tabulating data, analysis of variance was carried out. Results of the analysis of variance showed that the type of sugar, the concentration of sugar, and interaction between the two treatments had no significant effect on the total lactic acid bacteria on day 0 (without storing). However, after storing for 4, 8, and 12 days, the concentration of sugar had a very significant effect on the total lactic acid bacteria. Orthogonal comparison indicated that there were no significantly interactions between the two treatments. Total acid bacteria and their linear regression lines were presented at Figure 2, 3, and 4.

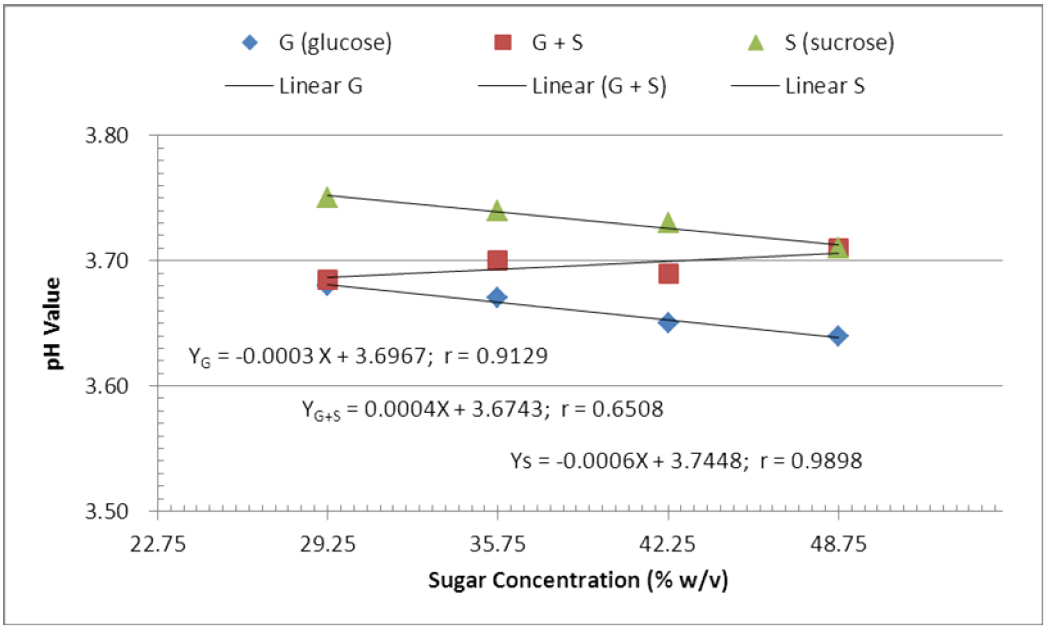

Figure 1. pH values of fermented turi-milks added with different types of sugar at concentrations of 29.25–48.75% and then stored at refrigerate temperature for 12 days.

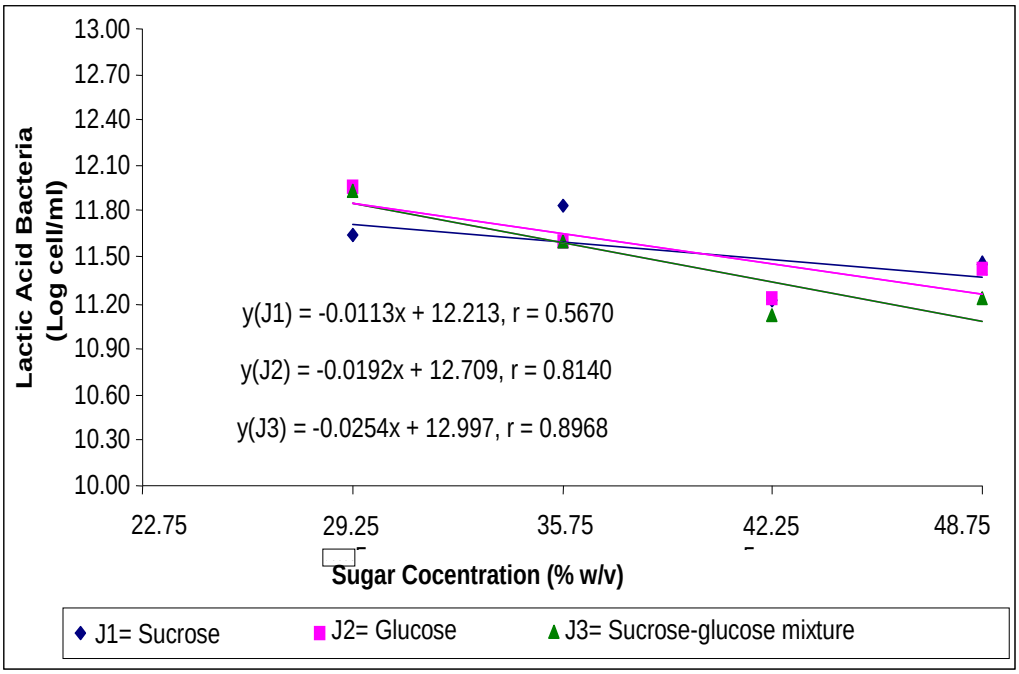

Figure 2. Total lactic acid bacteria of fermented turi-milks added with sugar at concentrations of 29.25 – 48.75% and then stored at refrigerate temperature for 4 days.

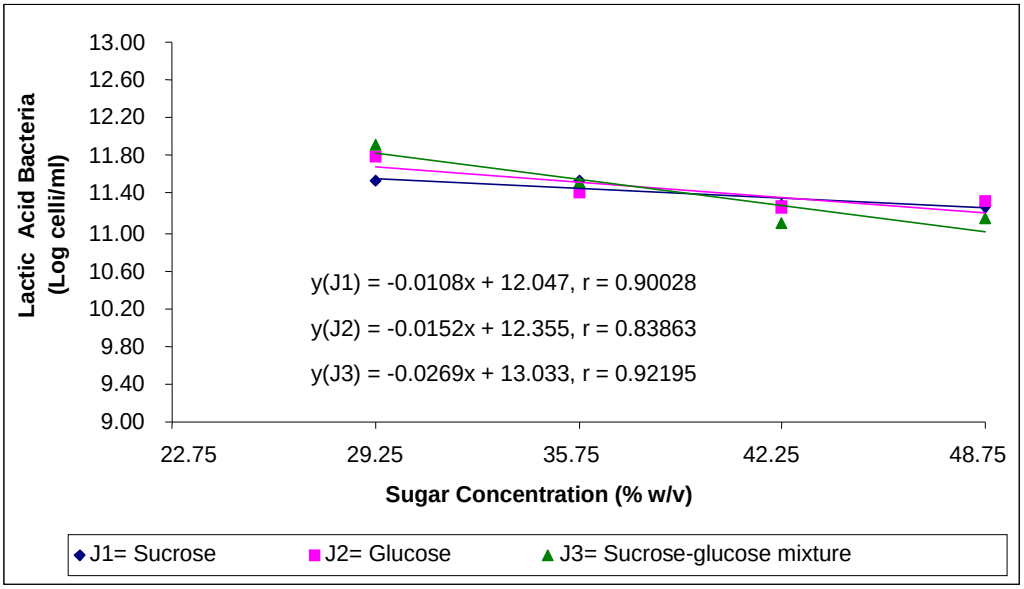

Figure 3. Total lactic acid bacteria of fermented turi-milks added with sugar at concentrations of 29.25 – 48.75% and then stored at refrigerate temperature for 8 days.

On day 0, total lactic acid bacteria was more than 10^{12} cfu/g (data was not presented). On the day 0, sugar was not able to inhibit the growth of lactic acid bacteria because of short exposure. The analysis of total lactic acid bacteria carried out immediately after the sugar was added to the fermented turi-milks.

The concentrations of sugar significantly decreased total lactic acid bacteria of the fermented turi milks after storing at refrigerate temperature for 4, 8, and 12 days (Figure 2, 3, 4). This phenomenon indicated that the bacterial growth rate decreased linearly with increasing sugar concentration. The higher concentrations of sugar,

the higher water removing from bacterial cells, so that the cells lacked of water and then died because of plasmolysis (separation of protoplasm from the cell membrane) (Membre et al., 1999).

As shown at Figure 2, 3, and 4, all fermented turi-milk that added with sugar and stored for 4, 8, and 12 days contained more than 10^{11} cfu/g total lactic bacteria. It means that the fermented turi-milks have fulfilled Codex standar for fermented Milks (Annonymous, 2010b). The standar stated that fermented milks should contain minimum 10^{7} cfu/g total microorganisms constituting the starter culture. Thus, all treatments (adding sugar at concentration of 29.25 – 48.75%, w/v) can be applied for producing fermented turi-milks.

Sensory evaluation of fermented turi-milk

After storing at refrigerate temperature for 12 days, fermented turi-milks were subjected to sensory evaluation to test their tase, flavor, and overall acceptability using a five point hedonic scale, where 1= dislike extremely, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately, and 5= like extremely. Result of the sensory evaluation was presented in bar diagrams and discussed descriptively.

Taste

Taste is an important parameter when evaluating sensory attribute of fermented milks. The milks might be appealing and having health benefit but without good taste, such milks are likely to be unacceptable. Results of taste evaluation were presented at Figure 5. Taste scores for fermented turi-milks - which were added with sugar - were 2.61 - 3.75 (out of 5 scale). Meanwhile, fermented turi-milks - which were not added with sugar – had a score of 1.36 - 1.64 (Figure 5).

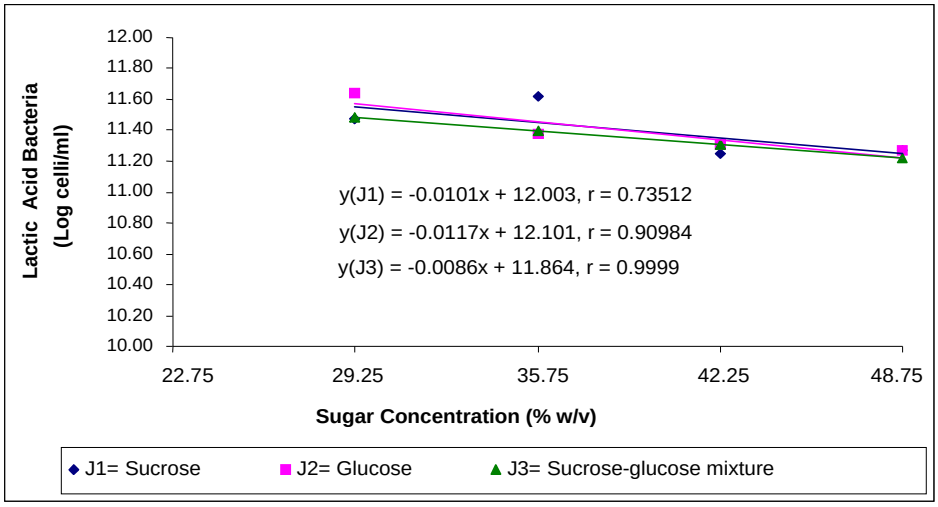

Figure 4. Total lactic acid bacteria of fermented turi-milks added with sugar at concentrations of 29.25 – 48.75% and then stored at refrigerate temperature for 12 days.

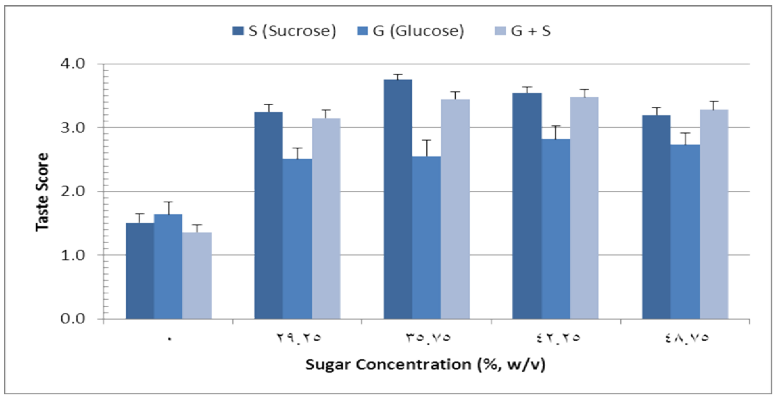

Figure 5. Taste scores of fermented turi-milks with or without sugar addition at concentrations of 29.25 – 48.75% and stored at refrigerate temperature for 12 days.

Adding sugar into fermented turi-milks was able to increase taste scores of the turi-milk (Figure 5). Without adding sugar, the fermented turi-milk went sour and panelists did not like it. By adding sugar, the fermented turi-milk went sweeter and the panelists were more like it. Adding sucrose into fermented turi-milks yielded the turi-milk having higher taste scores than that of adding glucose (Figure 5). Sucrose is sweeter than glucose because sucrose contains fructose, which is a sweet-tasting sugar ring that binds more tightly than glucose does to the sweetness receptor in the human mouth (Hendrickson, 2010). Joesten et al. (2007) stated that relative sweetess of sucrose, glucose, and fructose is 1.00, 0.74, and 1.17, respectively. The fermented turi-milk, which was added with 35.75% sucrose, had the highest taste score i.e 3.75 out of 5.00 scales. It means that the fermented turi-milk is the most favorable.

Flavor

Flavor is the sensory impression of a food or other substance taken into the mouth which stimulates one or both of the senses of taste and smell. Results of flavor evaluation were presented at Figure 6. Flavor scores for fermented turi-milks - which were added with sugar - were 3.01 – 3.40. Whereas, flavor scores for fermented turi-milks - which were not added with sugar - were 2.64 - 2.88 (Figure 6). It indicates that adding sugar into fermented turi-milks result in better flavor. This phenomenon agrees to the opinion of Tranggono et al. (1990). They said that the purpose of adding sweetness materials into foods or drinks were to improve their flavor so that sweetens taste could improve person's preference.

Lactic acid bacteria produce lactic acid and a small portion of citric, succinic, malic, and acetic acid, as well as carbonyl compounds such as acetaldehyde, diasetil, acetone and asetoin which acts as a flavor component (Ross et al., 2002; Salminen et al., 2004). The carbonyl compounds in high amounts especially diasetil are not desirable because it may cause *off flavor* in fermented beverages. Tamime and Robinson (1989) stated that natural yoghurt which has a high acidity level and a sharp flavor is less favored by consumers. The addition of sucrose would suppress the smell of carbonyl compounds (Tranggono et al., 1990); therefore, adding sucrose into fermented turi-milk results in favorable flavor.

Overall acceptance

Overall acceptance is affected by all sensory characteristics, such as taste, flavor, smell, texture, and appearance of food or drinks. Results of overall acceptance evaluation were presented at Figure 7. Overall acceptance score for fermented turi-milks which were added with sugar were 2.79 – 3.75. The scores for fermented turi-milks which were not added with sugar were 1.64 – 1.73 (Figure 7). Adding sugar into fermented turi-milks, results in higher overall acceptance. This is caused by the facts that sugar can improve taste and flavor of the fermented turi-milks. In addition, fermented turi milks which were added with sucrose have higher acceptance scores than those which were added with glucose or sucrose-glucose mixture (Figure 7) because sucrose is sweeter than glucose (Hendrickson, 2010).

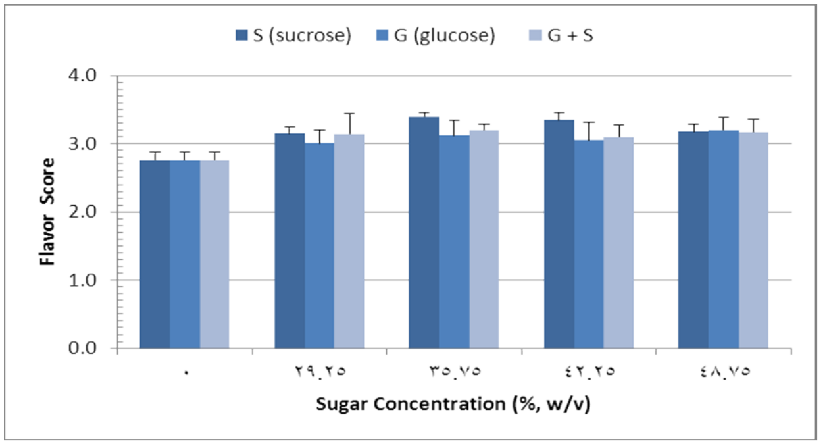

Figure 6. Flavor scores of fermented turi-milks with or without sugar addition at concentrations of 29.25 – 48.75% and stored at refrigerate temperature for 12 days.

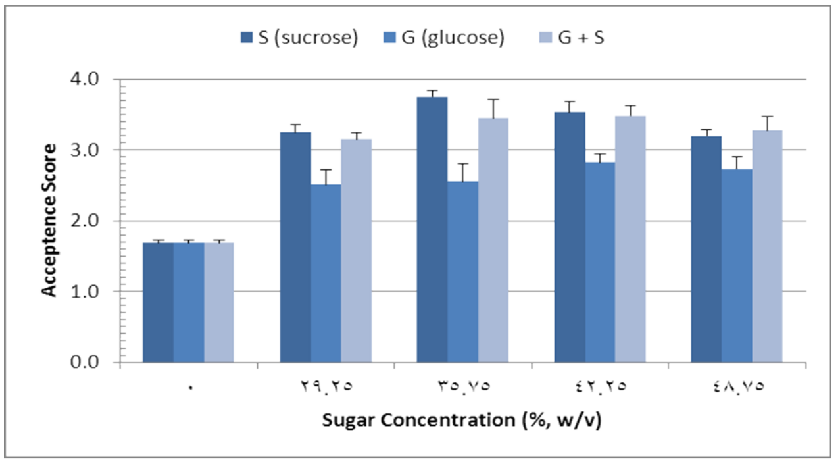

Figure 7. Overall acceptance scores of fermented turi-milks with or without sugar addition at concentrations of 29.25 – 48.75% and stored at refrigerate temperature for 12 days.

Selection of the best treatment

In this study, selection of the best treatment was based on the standard of fermented milks. Total lactic acid bacteria, pH, and sensory characteristics (taste, flavor, and overall acceptance) of the fermented turi-milks are compared to the available fermented milk standards. A treatment that fulfills the standards is considered as the best treatment.

The pH value of the fermented turi-milks which added with sugar has complied with the standar of fermented milks. All fermented turi-milks have a pH of 3.67 to 3.75 (Figure 1). Meanwhile, Australia New Zealand Food Standards Code - Standard 2.5.3 – stated that pH of fermented milk products is maximum 4.5 (Anonymous, 2011). Thus, all the fermented turi milks comply with the standar of fermented milks.

All the fermented turi-milks contain more than 10^{11} cfu/g total lactic acid bacteria (Figure 2, 3, and 4). Australia New Zealand Food Standards Code - Standard 2.5.3– stated that fermented milk products have to have minimum 10^{6} cfu/g microorganisms used in the fermentation (Anonymous, 2011). Meanwhile, Codex Standar for Fermented Milks – CODEX STAN 243-2003- stated that total microorganisms constituting the starter culture is minimum 10^{7} cfu/g and labeled microorganisms is minimum 10^{6} cfu/g. Thus all the fermented turi-milks produced in this research fulfill the standards of fermented milk products.

Since there are no standard for sensory characteristics of fermented milks, fermented turi-milks produced in this research are evaluated based on their overall acceptance scores to determine the best turi milks. Overall acceptance scores of all fermented turi-milks are presented at Figure 7. As shown at Figure 7, the fermented turi milk which was added with 35.75% sucrose has the highest overall acceptance score (3.75 out of 5.00). It means that the treatment (adding with 35.75% sucrose) is the best treatment for producing fermented turi-milk.

Based on the above discussion, all treatments (sucrose, glucose, and sucrose- glucose mixture at concentrations of 29.5, 35.75, 42.25, and 48.75% w/v) yielded fermented turi-milks which fulfill the available standards of pH and total lactic acid bacteria. Fermented turi-milks added with 35.75% sucrose had the best acceptance. Thus, the best treatment in this study was adding 35.75% sucrose into fermented turi-milk. The treatment yielded fermented turi-milk that had a pH of 3.71, total lactic acid bacteria of 3.62 x 10^{11} cfu/g, a taste scores of 3.75, a flavor score of 3.3, and an overall acceptance score of 3.75.

Conclusion

Adding sucrose at a concentration of 35.75% (w/v) was the best treatment for producing fermented turi milk. The fermented turi-milk had a pH of 3.71, a total lactic acid bacteria of 3.62 x 10^{11} cfu/g, a taste scores of 3.75, a flavor score of 3.3, and an overall acceptance score of 3.75 (like moderately).

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FOOD SCIENCE AND NUTRITION

Studies on preparation and preservation of lemongrass (*Cymbopogon flexuosus* (Steud) Wats) powder for tea

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Abstract

The leaves of six varieties of lemongrass at full maturity stage (whole and cut into 5 cm pieces) were blanched at 80°c for 1 min, dried, ground in to fine powder and analyzed for chemical constituents. The powder was stored in 200 guage plastic bags at ambient (31±4°C) condition for 6 months. The powder samples were drawn at 30 days interval and evaluated for chemical constituents and sensory quality. The lemongrass leaves of all varieties contained essential oil (1.20 to 4.40%), ascorbic acid (1.75 to 1.89 mg/100g) and total chlorophyll (7.49 to 10.76 mg/g) with reasonably good amount of ash (3.0 to 7.00%) on dry weight basis. The leaves and powder of *'Krishna'* variety contained maximum essential oil, ascorbic acid and total chlorophyll followed by *'Kaveri', 'Praman', 'Pragati' 'OD-19'* and *'CKP-25'* varieties. The overall acceptability score of tea extract with 1% powder of lemongrass was highest. During storage for 6 months, the essential oil ascorbic acid and total chlorophyll content were significantly decreased while ash content significantly increased. However, the interactions between treatments and storage period were nonsignificant. The sensory quality scores of powders of all the varieties were significantly decreased during storage. The powder of '*Krishna*' variety was rated first followed by '*CKP-25*', '*Kaveri*', '*OD – 19*', '*Pragati*' and '*Praman*'.

Key words: Lemongrass, Powder, Tea, Organoleptic taste, Chemical composition

Introduction

The lemongrass (*Cymbopogon flexuosus* (Steud) Wats) is a perennial grass belonging to family Graminaceae and grouped under genus *Cymbopogon*. It is of indigenous origin and is a medicinal and aromatic plant. It is locally known by different names such as 'Gawati Chah', 'Nibugrass', 'Puthiganda' etc. in different languages. The three species of lemongrass are found in India. *Cymbopogon flexuosus* is grown in East Indian States which is famous for its oil and has a good market. *Cymbopogon citrates* found in the West Indian States contain less citral. *Cymbopogon pendulus* in Jammu region contain higher high citral but its cultivation is limited.

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The lemongrass is grown in a wide range of soils and climatic conditions. It requires hot and humid climate with high rainfall and long sunlight and well-drained soil. It is originated in India and grown in states of Kerala, Karnataka, Tamil Nadu, Sikkim, Bengal, Madhya Pradesh, Arunachal Pradesh and Maharashtra. It is also grown in Brazil, Guatemala, Argentina, West Indies, Vietnam, Thailand, Shri Lanka and China. Wild species of lemon grass are found in tropical region of Asia, Africa and Latin America.

Lemongrass normally grows to a height of 1 to 1.5 m reaching 3 m at flowering. Leaves are linear, lanceolate, about 100-125 cm long and 1.0 to 1.5 cm broad. Inflorescence is a panicle, large, dropping lax, grayish to grayish green with purple tinge. It produces small blue flowers. Lemongrass is an open pollinated species and is adapted for cross pollination because of its protogynous nature (Mercy et al., 1979; Thomas, 1995).

India is traditionally the largest producer of lemongrass oil. However, the production has declined steadily over the years and export failed from 1800 tonnes in 1961-62 to 65 tonnes in 1994-

95 and today it is 400 tonnes (Dumbre and Bhoite, 1999). Latin America, China and Guatemala are the major producers of lemongrass essential oil in the world. The improved cultivars such as '*CKP-25*', '*Praman*', '*Pragati*', '*Kaveri*', '*Krishna*' and '*OD-19*' are grown in India (Pals et al., 1992; Kulkarni et al., 1999).

Lemongrass is well known for its essential oil which contains citral as the major constituent. The citral content varied from 44.3 – 91.4% to 79 – 91.5% (Kuriakose, 1995; Kulkarni and Ramesh, 1987). It is used as a starting material for synthesis of Vitamin A, in aromatherapy and in perfumery and flavourful grass, has therapeutic properties and is used internally as a medicinal tea. Oil is extensively used for scenting, waxes, polishes, deodorants. The leaves are used in teas in different countries as well as in culinary and cough treatment (Husain, 1994). The stalks are too tough to eat but, they can be chopped and powdered and add to flavour to fish or poultry sauces, grass leaves are used in curries and soup in Vietnam, Thailand and China. Lemongrass has medicinal use such as stomach smoother, also helps in reduction of cholesterol level. To make a tea from lemongrass leaves or powder and drink 1-4 cups per day to relieve congestion, coughing, bladder disorders, headaches, fever, stomach aches, digestive problems, diarrhea, gas, bowel sparms, vomiting, flu symptoms and to promote perspiration and as a possible cholesterol lowering agent. The leaves can also be dried and made into a powder for use in capsules. Powdered lemongrass is found in markets under the name "Sereh powder". The recipes such as lemongrass rice, sweet lemongrass blend, and lemongrass beef can be prepared from lemongrass.

The lemongrass has a very wide demand in nutritional, medicinal and flavouring industry. But it is not stored as fresh for long time at ambient condition because it rotten after long periods. Hence, lemongrass powder is preferred and it has huge demand in the world market. The price of the lemongrass powder is Rs. 80 to 100/kg and its oil is Rs. 300 to 500/kg. Traditionally, lemongrass powder is prepared by grinding the dried leaves. Lemongrass gives approximately 22.2 calories along with 4.59 g protein, 0.96 g sugar and 1.80 mg ascorbic acid per 100 g of powder. Also, lemongrass oil contains methyl eugenol, 15-20% (Sabti and Rao, 1987), geranyl acetate, 45-50% (Sabti and Rao, 1987 and Mathew et al., 1996), geraniol 49-80% (Kulkarni et al.,1992, 1996), bisabolo 37-38% (Thappa and Agrawal, 1989), Citronellol acetate and gerauyl acetate, 11.2 – 25.9% (Kulkarni et al., 1997), β-ocimene 17-20 % and V-terpineane, 8-10 per cent (Kulkarni et al., 1992) and elemiciu 53% (Sharma et al., 1999).

Owing to its medicinal value, excellent colour and flavour, it has a great potential in processing industry. It is, therefore, proposed to standardize the powder making technology from lemongrass and to study the organoleptic and chemical properties of lemongrass powder and the flavour stability during storage.

Materials and Methods

The lemongrass leaves of varieties such as *'CKP-25', 'Praman', 'Pragati', 'Kaveri', 'Krishna'* and *'OD-19'* at full maturity stage were obtained from the Medicinal and Aromatic Plants Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, India. The fully matured, healthy and uniform sized sound leaves were carefully harvested and brought to the laboratory for further experimental study. Most of the chemicals used in the present investigation were of analytical grade. Cross flow cabinet drier was used for drying of lemongrass leaves by keeping various treated samples in separate trays. Whelly Plant Grinding Mill from the Department of Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri was used to grind the dried lemongrass leaves samples. The leaves were cut into approximately 5 cm pieces according to the treatment details.

Fresh leaves (500 g) with various treatments given and oven dried at 50-60°C. After cooling, the samples were weighed again until constant weight was obtained. The weights were recorded as dry matter. The dried treated lemongrass leaves samples were ground into fine powder using Whelly Plant Grinding Mill for further chemical analysis and preparation of lemongrass tea for organoleptic evaluation.

The nitrogen, crude fiber, lipids, ash, total chlorophyll and essential oils content from various lemongrass leaves powder samples were estimated by AOAC (1990) methods. The total sugars content in was extracted in 80% ethanol solution (Nelson 1944). The vitamin C content was estimated by 2, 6, dichlorophenol-Indophenol dye visual titration method as described by Ranganna (1986). Total phenolics were determined by the method of Swain and Hills (1959).

Treatment details

The leaves at full maturity stage were harvested and then treated as follows for the preparation of powder.

a. Cutting treatments
    1. Whole leaves
    2. Cut into 5 cm pieces
b. Blanching treatments

1. Control
2. Blanching in 80°C hot water for 1 min
3. Blanching in 1% Na_2CO_3 at 80°C for 1 min

c. Drying treatment
1. Sun drying
2. Cabinet tray drying at 40°C

Preparation and packaging of powder

Evenly maturity, disease free and sound leaves of '*Krishna*', '*CKP-25'*, '*OD-19*', '*Praman*', '*Pragati*' and '*Kaveri*' lemongrass cultivar were selected. The leaves were washed with clean water and subjected to the above treatments like cutting, blanching and drying (Figure 1).

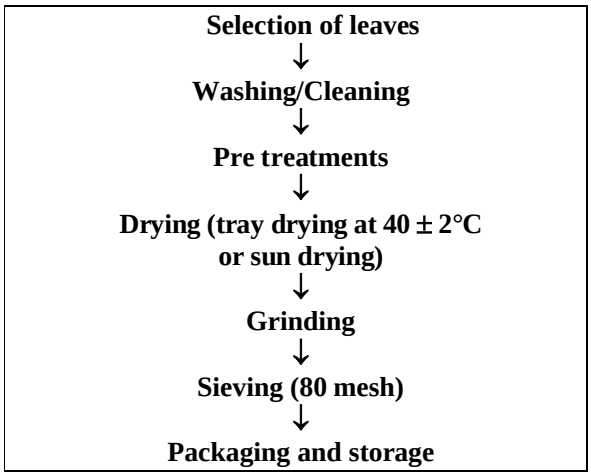

Figure 1. Flow chart for preparation of lemongrass powder.

The dried samples were ground into a fine powder and were sealed in 200 gauge LDPE bags and stored at ambient temperature. The chemical composition and organoleptic properties of lemongrass powder were tested by using different levels in the hot water or tea. The storage study was conducted up to 6 months.

Chemical analysis of powder

The powdered samples were chemically analyzed for essential oil, ascorbic acid, total chlorophyll and ash by the same procedure as described earlier for analysis of fresh leaves. The lemongrass powders were analyzed for chemical composition 60,120 and 180 days and for sensory qualities were assessed after 6 months of storage.

Sensory evaluation of powder

On the basis of highest amount of essential oil content '*Krishna'* variety was chosen for standardization of optimization of powder level for tea. Preliminary trials for standardization of level of lemongrass powder for preparation of tea were carried out using different levels viz. 0.5, 1.0, 1.5, 2.0 and 2.5 g/100 ml and heated upto boiling by adding 15% sugar. From these levels 1.0 g/100 ml lemongrass powder and 15% sugar level were standardized and used for further experimental study.

The sensory evaluation of powder stored at ambient condition was done at 180 days of storage by a panel of semi-trained judges as reported by Amerine et al. (1965) on 9 point Hedonic scale. At a time, six samples of powder were kept for evaluation and the average score of minimum five judges for each of colour of powder, colour of extract, aroma of extract, taste and overall acceptability was recorded. The experiment was planned by using Factorial Completely Randomized Design (FCRD) with 3 replications and the results were collected for chemical composition and sensory parameters. The results obtained were analyzed for the statistical significance according to the procedure as reported by Panse and Sukhatme (1967).

Results and Discussion

Physico-chemical characteristics of leaves

The leaves are dark green colored with an average weight of 3.5 to 4.5 g/100g leaves. Lemongrass leaves are linear, lanceolate, about 100-125 cm long and 1.0 to 1.5 cm broad. The fresh leaves contained average dry matter 43.59%, *'Pragati'* variety of lemongrass contained high dry matter 49.33% and low dry matter content was in *'OD-19'* variety of lemongrass as 39.93%. The nitrogen content in '*CKP-25*' variety of lemongrass was 0.98% and in *'Pragati'* was 0.49%. The crude fiber content of '*CKP-25*' variety was 85.5% and *'Praman'* was 77%. Average crude fiber content was 81.66%. The total sugars content in '*OD-19'* was 0.99% and *'Pragati'* 0.93%, average total sugars content was 0.96%. The ash content in *'Praman'* leaves was 6.33% and *'CKP-25'* is 3.00 average ash content was 5.33%. The ascorbic acid content in *'Krishna'* leaves was 1.89 mg/100 g and *'Kaveri'* and *'Pragati'* leaves content 1.75 mg/100 g, average vitamin C is 1.80 mg/100 g. The total chlorophyll content in '*OD-19*' leaves was 10.76 mg/g and *'Krishna'* leaves was 7.49 mg/g, average total chlorophyll is 8.93 mg/g. The total fat content in '*Krishna'* leaves is 4.80% and 'CKP-25' leaves was 1.40%, average total fat is 2.86%. The total phenolics content in '*Krishna'* leaves are 0.99% and 'CKP-25' leaves are 0.88%, average total phenolics are 0.941%. The essential oil content in '*Krishna'* leaves was 4.4% and '*CKP-25*' was 1.2%, average essential oil was 2.466% (Table 1). There for '*Krishna'* variety was used for preparation of lemongrass powder tea.

Best level of powder for preparation of lemongrass tea

Appropriate level of lemongrass powder for the preparation of extract/tea was determined using various concentrations such as control (green leaves 2.0%), 0.5, 1.0, 1.5, 2.0 and 2.5% in hot water containing 15% sugar. From this, best one was selected for further experiments on lemongrass tea preparation (Table 2). Considering the higher scores for colour, aroma of extract, taste and their overall acceptability score, 1.0% lemongrass powder level was found best for the preparation of lemongrass tea. Therefore, for further study 1.0% level of lemongrass powder was fixed.

Best treatment for preparation of lemongrass powders

From the six genotypes of lemongrass and drying treatments only one best treatment was selected for further storage study. For *'Krishna'* treatment Tray-whole-blanching in hot water, for '*CKP-25*' treatment Sun-whole-blanching in hot water for '*Kaveri'* treatment Tray-whole-blanching in hot water, for '*OD-19*' treatment sun-whole-blanching in hot water for *'Pragati'* treatment sun-whole-blanching in hot water and for *'Praman'* treatment sun-whole-blanching in hot water were found best. These treatments samples were further utilized for the storage study.

Sensory evaluation of fresh and stored lemongrass powder

The results indicated that the mean score for colour of powder was 7.84, colour of extract was 7.85, aroma of extract 7.69, taste of extract 7.94 and overall acceptability was 7.83. The colour of powder, colour of extract, aroma of extract, taste of extract and overall acceptability is better in '*Krishna'* variety powder which was 8.22 followed by *CKP-25* powder and '*Kaveri'* powder (Table 4). These results indicated that the varietal variation occur in the powder. This variation mainly contributes for the organoleptic properties of the lemongrass powder when used for the preparation of tea at specific concentration and conditions.

The results of sensory evaluation are reported in guava powder (Khurdiya and Roy, 1974; Ahire, 1989), mango powder (Dabhade and Khedkar, 1980), banana powder (Lal et al., 1989) and in sapota powder (Raut, 1999) are similar to these results.

Table 1. Chemical composition of six genotypes of lemongrass leaves (on dry weight basis).

Genotype	Dry matter (%)	Nitrogen (%)	Crude fibre (%)	Total fat (%)	Total sugars (%)	Ash (%)	Total chlorophyll (mg/g)	Ascorbic acid (mg/100 g)	Total phenolics (%)	Essential oil (%)
Krishna	40.00	0.84	82.50	4.80	0.98	4.66	7.49	1.89	0.99	4.4
CKP-25	41.66	0.98	85.50	1.40	0.95	3.00	7.71	1.82	0.88	1.2
Kaveri	45.00	0.77	78.00	3.60	0.97	7.00	10.12	1.75	0.90	3.1
OD-19	39.93	0.63	84.00	1.70	0.99	5.33	10.76	1.82	0.93	1.3
Pragati	49.33	0.49	83.00	2.60	0.93	5.66	9.32	1.75	0.98	2.2
Praman	45.66	0.70	77.00	3.10	0.94	6.33	8.20	1.82	0.97	2.6
Mean	43.59± 1.5	0.735± 0.0	81.66±0.3	2.86± 0.5	0.96±0.0	5.33 ±0.5	8.93±0.5	1.80±0.0	0.94±0.0	2.47±0.0
CD at 5 % (n=3)	4.282	0.223	1.071	1.446	0.0270	1.606	1.544	0.0606	0.0408	0.0521

Each value is the mean of three determinations.

Table 2. Organoleptic evaluation for optimization of lemongrass powder level for tea/extract.

Treatment	Colour of extract	Aroma of extract	Taste	Overall acceptability	Ranks
Control (Green leaves 2.0 %)	6.90	6.80	6.60	6.76	4th
(Dry powder) 0.5 %	7.00	6.60	7.20	6.93	2nd
1 %	8.10	8.40	8.20	8.23	1st
1.5 %	7.20	7.00	6.40	6.86	3rd
2.0 %	6.50	6.00	6.00	6.16	5th
2.5 %	5.60	5.40	5.60	5.53	6th

Each value is the average of ten trails.

Table 3. Effect of storage on chemical composition of lemongrass leaves powder (6 months storage).

Genotype	Essential oil (%)				Vitamin C (mg/100g)				Total Chlorophyll (mg/g)				Ash (%)			
	0	60	120	180	0	60	120	180	0	60	120	180	0	60	120	180
Krishna	3.00	2.70	0.27	0.20	1.48	1.33	1.21	1.08	3.09	2.93	2.85	2.73	7.00	7.60	8.00	8.70
CKP-25	0.90	0.70	0.50	0.50	1.55	1.41	1.28	1.15	3.38	3.25	3.12	3.03	10.00	11.35	11.5	11.9
Kaveri	2.30	1.57	1.47	1.40	1.62	1.48	1.35	1.21	5.82	5.75	5.67	5.60	7.20	7.78	8.49	9.00
OD-19	1.00	0.80	0.60	0.49	1.41	1.28	1.15	1.01	6.05	5.90	5.80	5.68	7.75	8.00	8.60	9.20
Pragati	1.90	1.60	1.20	1.00	1.48	1.35	1.21	1.08	4.93	4.80	4.68	4.59	8.00	9.00	9.50	10.28
Praman	2.20	1.80	1.60	1.48	1.68	1.55	1.41	1.21	4.28	4.15	4.06	3.97	5.93	7.00	7.50	8.00
Mean	1.88	1.53	1.27	1.18	1.54	1.40	1.34	1.12	4.59	4.46	4.36	4.27	7.65	8.45	8.93	9.51
Treatment/ Storage (n=3)	S. E. ±		C. D. at 5%		S. E. ±		C. D. at 5%		S. E. ±		C. D. at 5%		S. E. ±		C. D. at 5%	
T	0.034		0.095		0.023		0.065		0.024		0.068		0.048		0.135	
S	0.036		0.102		0.025		0.070		0.026		0.073		0.052		0.146	
T x S	0.089		N. S.		0.061		N. S.		0.064		N. S.		0.013		N. S.	

Changes in chemical composition of lemongrass powder during storage

The results indicated that there was a slight decrease in the essential oil content of the lemongrass powder with advancement of storage period. In general, essential oil content decreased from 1.88 to 1.18% (Table 3). The statistical analysis showed that there is a significant difference in the reduction of essential oil content with individual genotypes but the interaction effect genotypes vis. storage period showed non-significant effect. This indicates that lemongrass powder can be stored upto 6 months with good acceptability for the tea preparation at ambient condition. It is reported that, essential oil content decreased during storage in dried lemongrass leaves (Kulkarni, 1992).

The ascorbic acid of lemongrass powder decreased significantly in all varieties (Table 3). Mean decrease in vitamin C was from 1.53 to 1.12 mg/100 g. Ascorbic acid (Vitamin C) is one of the important vitamins needed in the human diet. It also play important role as an antioxidant. It increases nutritional value of the lemongrass powder. So it must retain in the lemongrass powder at higher level. Therefore, while giving various treatments to lemongrass leaves, care was taken for preservation of vitamin C in the lemongrass powder. But during storage there might be some oxidation due to presence of oxygen in the surrounding area of the lemongrass powder. The interaction between genotypes and storage period of lemongrass powder was non-significant. These results indicated that lemongrass powder can be stored upto 6 months in good condition. Ascorbic acid (vitamin C) might have been lost due to enzymatic oxidation in storage (Das and Dash, 1967).

The decrease in total chlorophyll content in all lemongrass powder during storage was observed. Mean of six genotypes and their storage period showed that the decrease in total chlorophyll was from 4.59 to 4.26 mg/g. The chlorophyll of lemongrass powder gives dark colour to the tea. Higher level of chlorophyll gives green colour to tea which is undesirable because it also affect the taste of tea. Therefore, optimum level or lower level of chlorophyll content in lemongrass powder is needed. While storage there might be oxidation of chlorophyll in natural environmental condition. This can cause reduction in the chlorophyll content of lemongrass powder. In the present study the chlorophyll content in lemongrass powder was at optimum level. There was neither very dark nor very faint colour to the tea prepared from them. Therefore, there was no significant difference in the colour parameters of tea prepared from six different genotypes. The statistical analysis of various genotypes and their storage period interaction showed non-significant effect. A decrease in total chlorophyll is reported in green teas during storage (Frederink, 1959).

The ash content of lemongrass powder increased significantly in all varieties as the storage period progressed. The average increase in ash content of lemongrass powder was from 7.64 to 9.51% (Table 3). Ash content of lemongrass powder non-significantly increased while stored upto 6 months at ambient condition. Ash contents all types of minerals and these minerals are not loosed during storage. There is no effect of environmental condition on the lemongrass ash content. The increase in the ash content in the lemongrass powder during storage might be due to reduction in the moisture content during storage as well as oxidation and reduction of other parameters

from powder. The increase in ash content is reported in chicory powder during storage (Ramalakshmi, 1994), dried spinach (Howard et al., 1962) and turmeric powder (Viasan et al., 1989).

Changes in sensory parameters of lemongrass powder during storage

The mean scores of sensory quality parameters of six varieties of lemongrass powders before storage were 8.10 for colour of powder, 8.16 for colour of extracts, 7.82 for aroma, 8.63 for taste and 8.10 for overall acceptability. On storage of powders for 6 months at ambient condition, the score for the colour of powder decreased. It decreased continuously from 8.10 to 7.40 as the storage period progressed (Table 4). The results indicated that the score for the colour of extract decreased during storage in all genotypes. It decreased continuously from 8.16 to 7.40 as the storage period progressed. The powder extract colour during storage period was decreased due to the oxidation or other biochemical reactions that occur during storage. Due to this reason while preparation of tea or extracting colour in hot water it was decreased. The decreasing rate of colour intensity was different for each genotype and might be due to their genotypic characteristics. The statistical analysis showed that the interaction between storage period and treatments were non-significant. The colour of extract slightly affected in green and black teas (Friderink, 1959) during storage at room temperature.

The results indicated that aroma of lemongrass powder extract score decreased from 7.82 to 7.50 throughout storage period. The variety '*Krishna*' powder showed score from 8.16 to 7.40 throughout the storage period. Aroma producing compounds are generally volatiles or they get combined with other biomolecules when samples are grinding or crushing for preparation of extract or powder. Mostly aromatic, flavors, flavonoids and essential oil gives aroma/smell to the lemongrass extract. Due to various treatments of cutting and powder making these aroma/smell forming compounds will be exposed to the natural environment. During storage period these compounds might get reacted with oxygen or other environmental condition and get reduced or defused. These are some of possibilities to reduce score of aroma of lemongrass powder's extract judged by semi-trained judges. The statistical results showed that there is non-significant difference in the reduction of aroma from all genotypes during 180 days storage period. The results revealed that the score for taste of extract decreased significantly from 8.63 to 7.43 during storage. The powder of '*Krishna*' variety gave better taste followed by '*Kaveri*' powder and '*Pragati*' powder. The taste organoleptic parameter mostly depend upon the compounds those are present in the extract/solution. In case of lemongrass powder's extract, there might be soluble sugars, free amino acids, chlorophyll, soluble proteins and other soluble metabolites. These all components may affect the taste of extract. During storage there might be oxidation, reduction and other reactions occur within these components as well as may be some complex formation. These all parameters may be responsible for the change in the taste of lemongrass powder's extract. The statistical analysis of the results showed that individual genotype or storage period reduced the taste score but their interaction was non-significant.

Table 4. Sensory quality of the lemongrass powder extracts (after 6 months storage at ambient condition).

Genotype	Colour of powder	Colour of extracts	Aroma	Taste	Overall acceptability	Rank
Krishna	8.26	8.20	8.16	8.25	8.22	1
CKP-25	8.20	8.03	7.80	7.80	7.96	2
Kaveri	7.80	8.20	7.40	8.20	7.90	3
OD-19	7.80	7.60	7.50	7.91	7.70	5
Pragati	7.40	7.68	7.75	8.03	7.72	4
Praman	7.62	7.40	7.53	7.45	7.50	6
Mean	7.19±0.32	7.17±0.27	6.98±0.22	7.23±0.32	7.14±0.25	-
C.D. at 5% (n= 5)	0.98	0.84	0.68	0.99	0.76	-

Each value is the average of five trails.

Figure in parenthesis are the mean score values of fresh lemongrass powder extracts.

There was a significant decrease in overall acceptability of lemongrass powder. The score decreased from 8.10 to 7.50 during the storage period. The overall acceptability of the extract is based on the organoleptic properties such as colour of powder, colour of extract, aroma of extract and taste of the extract. These parameters showed decreasing trend during storage period. Therefore, the overall acceptability also showed decreasing trend in all lemongrass genotypes. There was a variation in the rate of decrease in overall acceptability which is based on each genotype characteristic. The statistical analysis showed there is non-significant effect of storage period on the overall acceptability of lemongrass powder's extract when stored up to 180 days.

The cost of lemongrass powder did not include rent and transport charges, sales commission, local taxes etc. The results indicated that the cost of production of lemongrass powder at prevailing rate was in the range of Rs. 56.25 to 66.25/Kg.

Conclusion

The 1% powder level was better in quality for preparation of tea/extract as determined by the basis of 9 point Hedonic score method. Powder prepared from '*Krishna*' variety was found superior over other varieties of lemongrass in respect of essential oil, colour of powder, colour of extract, aroma of extract, taste and overall acceptability. All powder samples remained in good condition even after 6 months of storage in 200 guage polythene bags at ambient condition. Further studies on preparation of lemongrass powder on pilot scale and their mass consumer acceptability studies are needed for better utilization of lemongrass.

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Short Communication

FOOD SCIENCE AND NUTRITION

Microbiological quality and safety of dietary supplements sold in Saudi Arabia

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Abstract

The global market for dietary supplements has advanced in recent years capitalizing on the growing awareness of healthy living worldwide. Supplements provide enhanced nutritional levels for daily competitive performance. However, there is a need to explore the quality of dietary supplements as there are few studies related to this area. Therefore, the objective of this study was to determine the microbiological quality of dietary supplements in the local markets of Saudi Arabia. The total bacterial count, coliform, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* were included in this analysis. The 80 most popular supplements were tested in this study. Our results showed that microbial contamination was present in only nine products. The microbial level ranged from 1.69–8.43 Log CFU/mL. The higher level of total count (8.43 Log CFU/mL) and *S. aureus* (8.39 Log CFU/mL) were found in supplement glutamine. Amino acids, dynamisan, glucosamine sulfate, glucosaiene, creatine monohydrate, whey protein, and folate acid also showed the presence of bacterial contamination. Our findings suggested that improvements are needed in these supplements which were tested for microbiological contamination. These findings highlight the fact that a review of product safety and quality is becoming increasingly important for consumer health. This will help to ensure safe products available for today's savvy, health-conscious consumer.

Key words: Dietary supplements, Microbiological, Supplement quality, Supplement safety, Bacterial contamination

Introduction

The global market for dietary supplements has gained momentum in the past decade, and demand is increasing every year. Likewise, there is an increased awareness of the importance of supplement safety. The popularity of traditional herbs and botanicals is due in large part as these products are safe and consumable (Ravindran and Duraisankar, 2012). It is also increasingly evident that consumers are becoming more aware of functional foods and supplements as part of a balanced diet to ensure good health (Brink et al., 2005). There is a large variety of supplement types and brands available on the open market. Dietary supplements are products taken orally that contain one or more ingredients intended to supplement one's diet, and are not considered as food.

According to the United States' Dietary Supplement Health and Education Act (DSHEA) of 1994, dietary supplements are categorized as any "product" (other than tobacco) intended to supplement a diet and bears or contains one or more dietary ingredients (DSHEA, 1994). Examples of dietary ingredients include vitamin, mineral, herb or other botanical, amino acid, concentrate, metabolite, constituent, extract, or a combination of these ingredients. Supplements can be classified according to their function (muscle building, immune boosting, fuel providing), form (pills,

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powders, foods or drinks), availability (over-the-counter, mail order, Internet, multi-level marketing), and scientific merits of claims (well-supported, unsupported, undecided) (Burke et al., 2006). There is an increasing concern about the quality of dietary supplements as these products have been found to contain varying amounts of active ingredients in addition to contaminants and adulterants (Angell and Kassirer, 1998; Borins, 1998). However, there are very limited published information regarding the quality and safety of these products in Saudi Arabian markets. Therefore, the objective of this study was to determine the microbiological quality and safety of dietary supplements from Saudi Arabia.

Materials and Methods

Our preliminary data (Aljaloud et al., 2010) showed the presence of microbial contamination in dietary supplements collected from Saudi Arabia markets. Based on this study we further determined to analyze the microbiological quality of different dietary supplements available in the market. Eighty of the most popular supplements were collected. Each supplement was picked in duplicate from each store. Samples were then shipped to Greensboro, North Carolina, for microbial quality and product safety analysis.

Microbiological and safety quality test

From each commercial sample, three capsules or tablets were placed in 10 mL sterilized BHI broth and mixed thoroughly. Samples were then incubated at 37°C for 24 h to allow for microbial cell recovery if present. One milliliter from each sample was then withdrawn and diluted with 0.1% peptone water. The appropriate dilutions were plated onto duplicate non- selective and selective agar medium. To obtain the total bacterial count, samples were plated on Brain-heart infusion agar (BHI). To test for coliform and *E. coli*, samples were plated on Violet red glucose bile Agar (VRBGA) and MacConky agar, respectively. Similarly, samples were plated on Xylose lysine deoxycholate (XLD) agar for *Salmonella*, and Baird-Parker agar base (BPAB) for *Staphylococcus aureus* count (Aljaloud et al., 2009).

Results

In this study, we examined the microbiological quality of dietary supplements available in the markets in Saudi Arabia. We collected 80 different common dietary supplements in the city of Riyadh as listed in Table 1. We tested for the presence of total bacterial count, total coliforms, *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli*. The population level of bacteria ranged from 1.69 – 8.43 Log CFU/mL. The higher total count level (8.43 log) and *S. aureus* (8.39 log) were observed in supplement glutamine L. Similarly, other samples including amino acids, dynamisan, glucosamine sulfate, glucosaiene, creatine monohydrate, whey protein, and folate acid also showed the presence of bacterial contamination. In one product called 'pharmaton', we observed low total count (1.73 log) and *S. aureus* (1.69 log) respectively (Table 2).

Table 1. List of dietary supplements included in this study.

Sl. No.	Product Name
1	Creatine
2	Whey Protein
3	Glutamine
4	Multivitamins
5	Pyridoxin 40 (Vitamins B-6)
6	Amino acids
7	Vitamin B-12
8	Caffeine
9	GinkoBilobatabrts
10	Thiamine
11	Riboflavin
12	Vitamin B-1 300mg
13	Folate acid 5mg
14	Ephedrine
15	Dynamisan(Vitamins, Minerals and Amino acids))
16	Glutamine L
17	Ribose
18	Antioxidants
19	Vitamin C
20	GinkoBilobaKordel's
21	Vitamin D
22	Vitamin E
23	Iron tablets
24	Calcium tablets
25	Vitamin B- Complex
26	Glucosamine sulfate 750mg
27	Protein powder
28	Vita-C
29	Weight gainers
30	Fish oils
31	Omega 3
32	Omega 6
33	Methycobal 500mg
34	Ginseng products
35	Gingko biloba
36	Neurorubine forte
37	Red Bull energy drink
38	slimming
39	Coenzyme Q10
40	Guarana 250mg (Herbal Supplement)
41	Viforcit 1000mg

42	Guarana
43	WasserGlucoselcne
44	Creatine Monohydrate 700mg (dietary supplements)
45	Amino 1000mg (dietary supplements)
46	Evit(NaturalAntioxidants)
47	Pyridoxin
48	Navidoxin
49	pharmaton
50	Tri_B (Vitamin B1,B6,B12,Folic Acid)
51	Centram(multivitamin Formula)
52	Vitrite (multivitamin and Minerals)
53	Gineosan
54	Methycabal
55	Vito-p (multivitamin and Minerals)
56	Redoxon
57	Joint care (Oil)
58	Joint care (Powder)
59	Glucosamine sulfate 1000mg
60	Dynamisan with Ginseng (Complete dietary supplements)
61	Whey Protein isloates
62	Flax Oil 250mg Cold-Pressed&Unrefined dietary supplements
63	Arctic Cod Liver Oil237mg
64	Folate acid 1mg
65	Glucosaiene
66	Chitosan Glucomannan
67	Solotron 50 Plus Dietary Supplement 90 caplets Multivitamin
68	Gnc Triflex Dietary Supplement 120 Ea
69	Triple Strength Fish Oil Dietary Supplement 120 Softgels
70	Glucosamine 1000, Vegetarian Tablets 90
71	Vitamin E 400, Softgel Capsules 100
72	Natural Brand Lutein, 40mg, Softgel Capsules
73	Herbal Plus Grape Seed Extract, 100mg, Vegetarian Capsules
74	Gnc Melatonin 3 (Gnc)
75	Vitamin D
76	Green Tea Extract
77	Spirulina
78	glucosamine chondroitin joint health
79	Dynamic Health
80	Calcium Complete with Magnesium, Softgel

Table 2. Bacterial population (Log CFU/mL) present in different supplements.

Product	Bacterial population (Log CFU/mL)	
	Total count	*S. aureus*
Amino acids	7.54	7.38
Dynamisan	5.9	4.93
Glutamine L	8.43	8.39
Glucosamine sulfate	7.53	7.66
Glucosaiene	7.47	7.2
Pharmaton	1.73	1.69
Creatine Monohydrate	4.85	4.98
Whey Protein	6.2	5.54
Folate acid 5mg	4.6	4.36

Discussion

Quality assurance and standardization are two important key factors in the food products development. To determine the microbiological quality and safety of dietary supplements, tests need to be conducted to ensure their microbiological quality. Medicinal plant materials should be entirely free from visible signs of contamination by molds or insects, and other animal contamination. Medicinal plant materials carry a great number of bacteria and molds often originating in soil, while a large range of bacteria and fungi occur naturally on the surface of herbs. Harvesting, handling and production may also cause additional contamination and microbial growth in such source of plant ingredients. Selection of plant materials based on quality, standardization, methods of preparation, and enforcement of regulation regarding appropriate labels are measures which will improve the quality and acceptance of herbal preparations as therapeutic agents (Kuruvilla, 2002).

Okunlola et al. (2007) studied the microbial quality of different medicinal products in Southwestern Nigeria which included 21 different brands of herbal medicine. This shows that among the tested products, microbial load varied considerably. Over forty-seven percent of the samples showed contamination with *E. coli*; 33% with *Salmonella*; 71.4% with *Staphylococcus aureus;* and 57.1% were contaminated with fungi. Significant contamination with bacteria and fungi was reported during the investigation of the microbial quality of herbal medicines collected

from the shops in the Nelson Mandela Metropolis. The presence of *Salmonella, and, E. coli* in herbal powder and tablets have been also reported earlier (Ravindran and Duraisankar, 2012). In our study, out of eighty dietary supplement samples tested, nine products showed the presence of coliform, *E. coli*, *Salmonella* and *Staphylococcus aureus*. Hamilton-Miller et al. (1999, 2002) reported that out of 30 probiotic suppelmenbts tested, 18 contained species other than those on label indicating poor standard of supplements contrary to the claim. Authors have also reported the presence of potentially pathogenic species such as *E. faecium.* Similarly, Hamilton-Miller et al. (1999) and Barros et al. (2002) have found pediocci as contaminants in several probiotic supplements. The presence of such bacterial strains could increase the risk of bacterial outbreaks. Therefore, these results along with our findings emphasize for the need to improve the quality of dietary supplements sold in the markets.

Conclusions

From this study we can conclude that there are some contaminations in supplements sold in Saudi Arab markets. The presence of microraginsm (*E. coli, Salmonella, S. aureus*) could be potentially harmful for human health. Improvements on the presence of such microbiological contamination by good manufacturing practice for handling, packing and storage is needed. This could be done by monitoring the manufacturing plants with trained quality assurance personnel.

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PLANT SCIENCE

Genetic variation between *Xylocarpus* spp. (Meliaceae) as revealed by Random Amplified Polymorphic DNA (RAPD) markers

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Abstract

The genetic diversity of *Xylocarpus* a tree mangrove species was assessed. Three species viz., *X. granatum, X. moluccensis* and *X. mekongensis* were collected from different localities of the states of Tamil Nadu and Andhra Pradesh in India. These three species showed high degrees of phenotypic variation in the vegetative parts such as leaf, bark and root. To assess the variations at the molecular level within the three species of *Xylocarpus* a RAPD technique was used. A total of 283 DNA fragments were generated by 25 random primers, with an average of 11.32 easily detectable fragments per primer. A higher similarity was observed between *X. mekongensis* and *X. moluccensis*, whereas *X. granatum* and *X. mekongensis* showed lower similarity. *X. granatum* and *X. mekongensis* had a variation of more than 79 %. On the other hand, *X. mekongensis* and *X. moluccensis* seem to be closely related with 35% variation. These variations may be due to its genotypic variation, isolated distribution and adaptation to dissimilar edaphic and environmental factors. All the three species of *Xylocarpus* are related at various degrees. These results will be helpful in future to assess the existing interspecific genetic polymorphism in *Xylocarpus* species and to design strategy for their conservation.

Key words: Genetic diversity, *Xylocarpus*, RAPD

Introduction

Mangroves are unique plant communities inhabiting the estuarine and inter-tidal regions of both tropical and subtropical coasts. The total number of exclusive or true mangrove species in the world is 68 and they belong to 27 genera (Duke, 1992). The genus, *Xylocarpus*, belonging to the family Meliaceae has three distinct species, *viz*., *X. granatum* Koen., *X. moluccensis* Lamk. and *X. mekongensis* Pierre. These species are distributed in tropical tidal forests of old world, typical mangrove habitats or in sandy or in coastal habitats spread from Africa to Australia including India and Malayan Archipelago (Tomlinson, 1986). In India, these species were recorded from Andaman Islands, coastal line of Orissa, Andhra Pradesh and Pichavaram mangrove forest of Tamil Nadu in the East coast of India. *X. granatum* is distributed in West coast mangroves e.g. Maharashtra of India. *X. granatum* and *X. mekongensis* are moderately sized trees with a well-developed woody trunk, whereas *X. moluccensis* is a medium-sized crooked, much-branched evergreen tree up to 10 m tall. They are found on the fringes of backwater creeks. Usually this taxon is associated with *Avicennia, Excoecaria, Acanthus, Rhizophora* and *Bruguiera* (Raju, 2003). The bark of *X. mekongensis* is rich in tannin and is used for tanning heavy hides, toughening fishing-nets and dying cloth. The wood is of good quality and used for boat building, nails, house-posts, small objects, furniture and firewood. The seeds have medicinal properties. Molecular markers like random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism are extensively used to quantify the inter-specific, intra- specific and inter-generic variability in different plant groups and crop varieties (Chalmers et al., 1994; Lin et al., 1996; Garcia Mas et al., 2000; Sharma et al., 2000; Mukharjee et al., 2003; Begum et al., 2013). Parani et al. (1997) identified the parentage of *Rhizophora* hybrid, by establishing its maternal status using molecular markers such as RAPD and RFLP. Jian et al. (2004) analyzed the variation in inter simple sequence repeats (ISSR) in mangrove and non-mangrove populations of *Heritiera littoralis.* In 2004, Mukherjee and his

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associates studied the genomic relationship among nine mangrove and two non-mangrove species of India belonging to the family Rhizophoraceae and proved that mangroves and non-mangroves are related distantly. Su et al. (2006) studied genetic variation in the mangrove, *Lumnitzera racemosa*.. Triest (2008) reviewed the uses of dominant markers such as RAPD and AFLP for identifying the mangroves and studying their relationship. Jugale et al. (2009) assessed genetic diversity in intra and inter-populations of *X. granatum* using ISSR markers and showed that variation exists at the phenotypic level but the genetic variability among the populations of *X. granatum* is very low. The molecular markers are phenotypically neutral having no epistatic and developmental effects. Moreover, they can detect the variation in both coding and non-coding regions of the genome. Among the several types of markers RAPD markers have been successfully proven to distinguish the variations. There are no attempts made to study the genetic diversity of three mangrove species of the genus, *Xylocarpus* in peninsular India.

In the present study the genus *Xylocarpus,* a mangrove species has been assessed for genetic diversity. Three species viz., *X. granatum*, *X. moluccensis* and *X. mekongensis* were collected from different localities Tamil Nadu and Andhra Pradesh in India. These three species showed higher degrees of phenotypic variation in the vegetative parts such as leaf, bark and root. To assess the variations at the molecular level within three species of *Xylocarpus*, the RAPD technique was used. RAPD markers may help to find out the difference at the molecular level between these species, and which in turn will prove useful to explore the adaptation of these three species in different habitats.

Material and methods

Plant material and DNA extraction

The seeds of *X. granatum* and *X. moluccensis* were collected from Kothapalem (11º 51′ N and 80º 54′ E) of Andhara Pradesh and *X.mekongensis* from Pichavaram (11º 27′ N and 79º 47′ E) of Tamil Nadu. The physical characteristics of sampled location given below in Table 1. The seeds were collected during the monsoon season in the month of November- January (2010-2011). The seedlings of the 3 species (*X. granatum, X. mekongensis* and *X. moluccensis)* were raised in the Botanical garden, Department of Botany, Annamalai University. The morphological features of these three species were recorded (Table 2 and Figure 1) Genomic DNA was isolated from freshly collected leaves using the CTAB (cetyl trimethyl ammonium bromide) method (Saghai-Maroof et al., 1984) with some modifications. 0.5 g of young leaf tissue was ground in liquid Nitrogen to fine powder using sterile pestle and mortar and suspended in 750 µl of preheated 2% CTAB buffer (2% CTAB; 0.1 M Tris pH 8.0; 20 mM EDTA; 1.4 M NaCl; 2% Polyvinylpyrrolidine-40 and 1% β-mercapto ethanol). The suspension was incubated at 65°C for one hour with occasional inversion. An equal volume of chloroform: isoamyl alcohol (24:1) mixture was added to the suspension and centrifuged at 10,000 g for 15 minutes. The aqueous phase was transferred to a new microfuge tube and extracted with 0.2 volumes of 5% CTAB buffer and equal volume of chloroform: isoamyl alcohol (24:1) mixture at 10,000 g for 15 minutes. The Chloroform: IAA extraction step was repeated twice. The aqueous phase was transferred to a fresh tube containing 0.6 volumes of ice-cold isopropanol and incubated overnight at -20°C and subsequently, centrifuged at 10,000 rpm for 15 minutes, to recover the nucleic acids. The pellet was washed with 70% ethanol and air dried and dissolved in TE buffer (10 mM Tris; 1 mM EDTA, pH 8.0). The extracted DNA was quantified by using Nanophotometer (IMPLEN, GmbH, Munich, Germany) and diluted to 15 ng/ µl for PCR amplifications.

Evaluation of genetic diversity

The 25 RAPD 10-mers used (Table 3) were selected from among 134 RAPD primers (obtained from Operon Technologies USA) in a preliminary test for oligos that amplified numerous discrete fragments. RAPD profile of five primers across the three species is depicted in Figure 2. Every 15 µl reaction volume consisted of 15 ng of genomic DNA, 1.5 µl of 2mM each dNTPs, 1.5 µl of 10x Taq DNA Polymerase assay buffer, 1.8 µl of 15mM $MgCl_2$, 5 µM RAPD primers and 1 U Taq DNA polymerase (Fermentas). PCRs were performed using a in an Eppendorf Thermocycler and involved an initial denaturation step (94°C, 3 min), 40 amplification cycles (each 94°C, 30 s; 37°C, 30 s and 72°C, 60 s) and a final extension step (72°C, 15 min).

Table 1. Physical characteristics of sampled locations.

Location	Physical Characteristics		
	Soil pH	Salinity (ppt)	Soil type
Kothapalem (Andhra Pradesh)	7.5 – 8.3	6 – 14	Saline swampy
Pichavaram (Tamil Nadu)	7.8- 8.4	8 – 24	Fine and coarse silt

Table 2. General morphological characters to identify the 3 species of *Xylocarpus.*

Morphological characters	*X. granatum*	*X. mekongensis*	*X. moluccensis*
Leaves	Paripinnate leaves, light green in colour with a rounded apex, Elliptic /obovate leaflets	Paripinnate leaves having 1, 2 or 3 pairs of leaflets with pointed apex	Leaves paripinnate, long, glabrous; leaflets upto 12 × 5 cm, elliptic, oblong-elliptic or obovate-elliptic, apex usually rounded.
Bark	Trunk surface is pale, smooth with its thin bark peelings in flakes or patches	Trunk surface is dark brown, fissured with the bark peelings in long thick narrow strips	Bark smooth and yellowish or brown and green with flaking
Root	Horizontal cable roots develops into ribbon like plank roots	Vertical conical laterally compressed knee roots or pneumatophores present	Surface roots laterally compressed and forming a spreading network of ribbon like pneumatophores.
Flowers	Inflorescence is regular with 3 flowered cymes, Flowers small and white in colour	Inflorescence is lax upto 10 cm long or more, flowers creamy white in colour	Flowers whitish or pale pink, in lax racemes of 2-3 lowered cymes.
Fruit	Large globose upto 20-30 cm across	Subglobose 8-12 cm across	Large 10-15 cm across

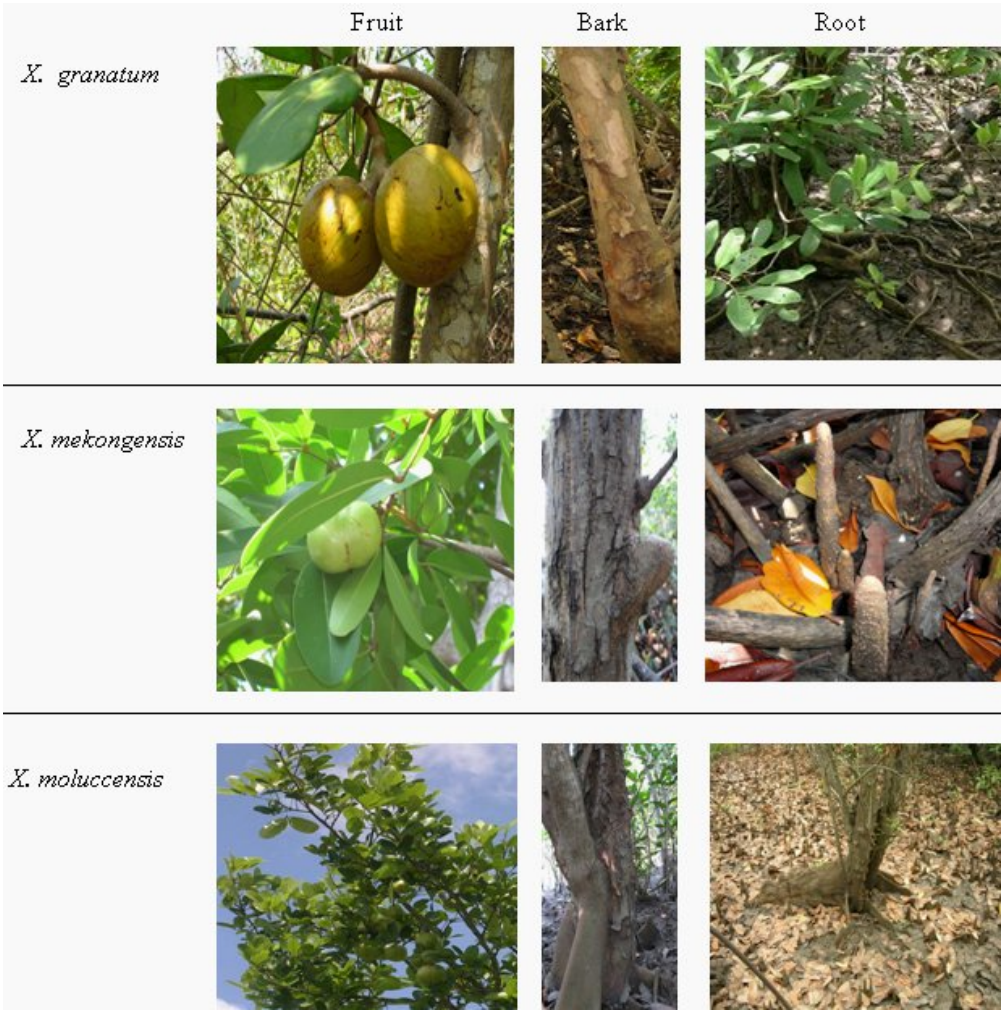

Figure 1. Morphological variations among the three species of *Xylocarpus.*

Table 3. Details of the random primers used for the genetic relationship studies across three species of genus *Xylocarpus.*

Marker	Sequence (5' – 3')	Total no. of loci amplified	Range of amplicons (bp)
OPK04	CCGCCCAAAC	15	240 – 1480
OPK08	GAACACTGGG	7	500 - 3000
OPL01	GGCATGACCT	16	250 - 3500
OPL05	ACGCAGGCAC	12	550 - 2000
OPL07	AGGCGGGAAC	8	600 - 1900
OPL18	ACCACCCACC	8	680 - 2500
OPL19	GAGTGGTGAC	14	600 - 6000
OPM10	TCTGGCGCAC	14	500 - 3700
OPM11	GTCCACTGTG	3	750 – 2250
OPM20	AGGTCTTGGG	11	300 - 2500
OPQ20	TCGCCCAGTC	12	350 - 3000
OPT02	GGAGAGACTC	15	400 - 3000
OPW05	GGCGGATAAG	18	250 - 3000
OPW08	GACTGCCTCT	11	400 - 6000
OPX01	CTGGGCACGA	14	400 – 2500
OPX02	TTCCGCCACC	12	400 - 2500
OPX03	TGGCGCAGTG	16	350 - 3000
OPX15	CAGACAAGCC	12	300 - 2250
OPY01	GTGGCATCTC	8	625 - 3500
OPY02	CATCGCCGCA	7	470 - 2000
OPY03	ACAGCCTGCT	10	280 – 1520
OPY05	GGCTGCGACA	7	580 - 1500
OPY06	AAGGCTCACC	7	550 - 1600
OPZ14	TCGGAGGTTC	11	525 - 1800
OPZ15	CAGGGCTTTC	15	400 - 2200
Total		283	-

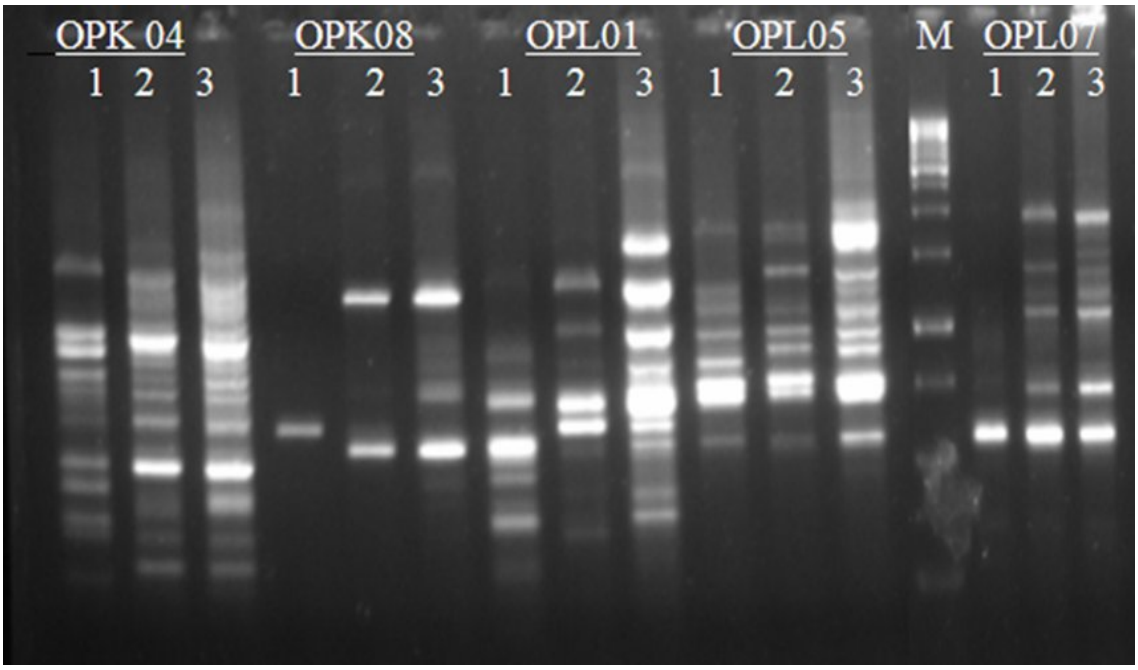

Figure 2. RAPD profile of three species of *Xylocarpus.*

Table 4. Details of the RAPD analysis with the three *Xylocarpus* species.

Marker	Total No. of loci obtained			No. of polymorphic loci			% polymorphism		
	Xg	Xme	Xmol	Xg	Xme	Xmol	Xg and Xme	Xme and Xmol	Xg and Xmol
OPK04	8	8	9	6	6	7	85.71	45.45	69.23
OPK08	1	3	6	1	3	6	100.00	50.00	100.00
OPL01	6	6	11	4	4	9	81.81	69.23	78.57
OPL05	7	7	9	3	3	5	60.00	45.45	33.33
OPL07	2	6	7	0	4	5	66.66	37.50	71.42
OPL18	3	4	6	2	3	5	83.33	57.14	71.42
OPL19	8	7	7	6	5	5	84.61	25.00	84.61
OPM10	8	6	6	8	6	6	100.00	0	100.00
OPM11	1	3	3	0	2	2	66.66	0	66.66
OPM20	5	6	7	4	5	6	90.00	14.28	90.90
OPQ20	11	2	3	10	1	2	91.66	33.33	83.33
OPT02	10	3	12	8	1	10	81.81	75.00	53.33
OPW05	11	6	11	10	5	10	93.75	45.45	77.77
OPW08	9	1	4	8	0	3	88.88	75.00	81.81
OPX01	9	10	11	3	4	5	61.53	9.09	57.14
OPX02	1	11	2	0	10	1	90.90	91.66	50.00
OPX03	3	14	6	2	13	5	93.75	66.66	71.42
OPX15	6	6	6	6	6	6	100.00	0	100.00
OPY01	5	6	6	2	3	3	62.50	0	37.50
OPY02	2	6	4	1	5	3	85.71	33.33	80.00
OPY03	4	9	6	2	7	4	70.00	33.33	75.00
OPY05	5	5	5	2	2	2	71.42	0	57.14
OPY06	6	5	5	2	1	1	42.85	0	42.85
OPZ14	7	8	4	7	8	4	63.63	50.00	100.00
OPZ15	7	11	9	5	9	7	73.33	18.18	85.71
Total	145	159	165	102	116	122	-	-	-
Average	5.8	6.36	6.6	4.08	4.64	4.88	79.62	35	72.76

Xg = *X. granatum* Xme = *X. mekongensis* Xmol = *X. Moluccensis*

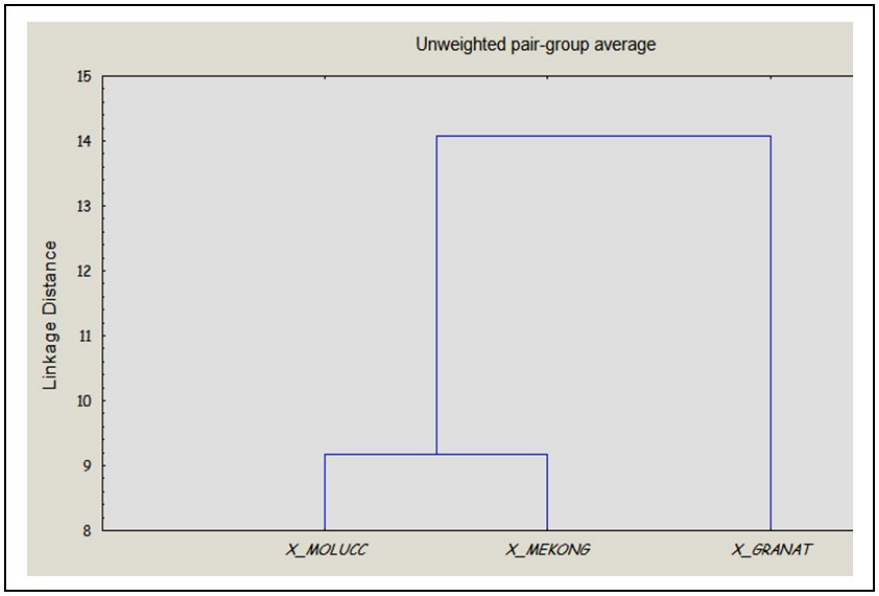

Figure 3. UPGMA dendrogram showing the relationship among three species of *Xylocarpus*.

Data analysis

The amplified products were separated on 1.5% (w/v) agarose gels by electrophoresis in 1X TBE buffer and visualized under UV light after ethidium bromide staining. To confirm the reproducible amplification of scored fragments, all amplifications were repeated twice. All the visible RAPD fragments were counted for each primer (Table 4) and robust polymorphic bands were scored as present (1) or absent (0) for each sample. For each primer, the number of polymorphic bands was calculated. The statistical analysis was carried out using the STATISTICA program (version 4.5 Stat soft Inc, USA). A phylogenetic dendrogram (Fig 3) was obtained by cluster analysis following the Unweighted Pair Group with Arithmetic Mean (UPGMA) method.

Results and Discussion

A total of 25 random primers were used for RAPD analysis with the 3 species of *Xylocarpus.* Majority of these primers showed polymorphic bands across the three species. PCR amplification using the genomic DNA of three species of *Xylocarpus* with 25 RAPD primers produced a total of 283 bands, of which, 242 bands were polymorphic. The size of the amplicons ranged from 240 to 6000 basepairs. Mangroves are constantly subjected to physiological stress caused by fluctuating growing conditions (Chapekar, 1994). Despite such extremes, they have successfully colonized suitable areas by developing morphological, physiological and reproductive adaptations (Clough et al., 1982; Clough et al., 1994; Saenger, 1982). Therefore depending on the genetic architecture of these species and their edaphic preferences and adaptations, different species are likely to display varying degree of polymorphism. Present observations on *Xylocarpus granatum* do support this presumption. In the three species, a maximum of 18 loci was obtained with the primer-OPW5, while the primer OPM11 resulted in a minimum of 3 loci. The total RAPD loci between the *Xylocarpus* species for individual primers differed according to the genomic characteristics of the species and the total number of amplicons resolved was 165 in *X. moluccensis*, followed by 159 in *X. mekongensis* and 145 in *X. granatum*. Ge and Sun (1999) studied genetic variation within and among populations of *Aegiceras corniculatum.* They recorded very low variation and low gene differentiation among populations. Huang et al. (2008) assessed interspecific and inter-population variation in three species of *Ceriops* and recorded low-genetic diversity at population level.

The average number of bands per primer varied from 5.8 to 6.6 in the species of *Xylocarpus*. The number of polymorphic loci ranged from a minimum of 102 in *X. granatum* to a maximum of 122 in *X. moluccensis* with an average ranging from 4.08 to 4.88 with the 25 random primers. It has been reported by Mukharjee et al. (2005) that nine members belonging to Rhizophoraceae formed a single cluster with RAPD analysis. The maximum average percent polymorphism of 79.62% was observed between the species of *X. granatum* and *X. mekongensis*, whereas the minimum DNA polymorphism of 35% existed between *X. mekongensis* and *X. moluccensis* and it was 72.76% with *X. granatum* and *X. moluccensis.* The cluster analysis of the RAPD profile with 25 primers following the method of Unweighted Pair Group with Arithmetic Mean (UPGMA) revealed that the 3 species of *Xylocarpus* clustered into two distinct branches of a single tree. *X. mekongensis* and *X. moluccensis* clustered together with a linkage distance of 9.2, whereas *X. granatum* is 4.8 linkage distance away from the above two species. *Xylocarpus mekongensis* and *Xylocarpus moluccensis* formed a single cluster while *X. granatum* formed different cluster. All the three species of *Xylocarpus* are related at various degrees, while *X. granatum* is distantly related with *X. mekongensis* and *X. moluccensis*. The *X. mekongensis* and *X. moluccensips* seem to be closely related and have 35% variation. This result coincides with the morphological and floristic variation exists among the species. *Xylocarpus granatum* is a critically endangered species of mangrove along the Indian coastline disappearing from many locations and represented by few individuals (Jugale et al., 2009). Loss of individuals or populations at certain locations may not cause immediate loss in genetic diversity, but more damage may occur in terms of long term genetic consequences due to the reduced numbers of populations and smaller population sizes. These variations may be due to isolated distribution and adaptation to dissimilar edaphic and environmental variation exists within the mangroves of Peninsular India.

In conclusion, our results demonstrate that molecular markers provide an effective tool to access the existing interspecific genetic polymorphism in mangrove species and to design their conservation strategy. The Mangrove genus *Xylocarpus* having high genetic variability may be

due to varied edaphic and climatic conditions of various mangroves localities.

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ANIMAL SCIENCE

Effects of fish oil on the production performances, polyunsaturated fatty acids and cholesterol levels of yolk in hens

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Abstract

This study was conducted to show that dietary supplementation of fish oil, effects growth performance, egg quality and decreases yolk cholesterol in laying hens. One hundred twenty Bovans white hens at their 23rd week of age were housed individually in cages in an open sided house. The birds were kept under a 16 hr light: 8 hr dark lighting schedule (lights on between 06:00 and 22:00 hr). Birds were randomly divided into five dietary treatments and 3 replicates in each (8 birds per replicate); the first treatment group was control and given 0% fish oil + 5% vegetable oil, the second treatment given 1.25% fish oil +3.75 % vegetable oil, the third treatment given 2.5% fish oil + 2.5% vegetable oil, the fourth treatment given 3.75% fish oil + 1.25% vegetable oil and the fifth treatment given 5% fish oil + 0% vegetable oil. The birds were raised for 12 weeks from 23 weeks of age to evaluate the effect on growth, egg quality, yolk cholesterol and linoleic and linolenic fatty acids content in yolk. Body weight gain was increased significantly ($P \leq 0.05$) by 9.2% in birds fed diets content 3.5% fish oil, as compared with those fed on control diet and feed intake was decreased significantly ($P \leq 0.05$) by 26.4% in birds fed diets content 5% fish oil, as compared with those fed on control diet. Also, egg weight was decreased significantly. Due to fish oil egg quality were improved. Interestingly, yolk cholesterol was decreased significantly ($P \leq 0.05$) by 14.5% in birds fed diets content 3.5% fish oil, as compared with those fed on control diet. Furthermore, it was observed significantly increase ($P \leq 0.05$) in linolenic fatty acid content in yolk by 30.5% in birds fed diets content 3.5% fish oil, as compared with those fed on control diet. In conclusion, feeding fish oil deceased feed intake, egg weight and egg production on the other hand, increased unsaturated fatty acids and cholesterol content in yolk in laying hens.

Key words: Omega-3 fatty acids, Hens, Egg quality, Cholesterol, Performance

Introduction

Eggs are considered as an important part of human feeding since the dawn of recorded history. Good taste and numerous applications in preparing a wide variety of foods lead to increase the egg consumption in the world year by year. The egg functional proteins have been recognized as one of the highest quality proteins in digestibility as well as amino acid composition. Hen eggs have been evaluated as a source of essential fatty acids, and several vitamins and minerals thus, daily intake of hen eggs supply nearly the amount recommended of our daily allowance of such materials. These advantages qualify hen eggs to be one of the promising functional foods in the coming decades according to their relation to the human health. Functional foods may be defined as foods or food ingredients that may enhance health through their provision of a physiological benefit beyond traditional nutrients. Examples of functional foods include foods that contain specific minerals, vitamins, dietary fiber, foods with added biologically active fatty acids or substances such as phytochemicals or other antioxidants and probiotics. Being a functional food has been revealed new trends in egg production like designer eggs and specialty eggs (Eid, 2005). Omega-3-polyunsaturated fatty acids (n-3PUFA) play an important role in human nutrition since they help to reduce the incidence of such life-style diseases as coronary artery diseases, hypertension and diabetes, as well as certain inflammatory diseases as arthritis and dermatitis (Simopoulos, 2000). These diseases are an increasing problem in countries of the Middle East and North Africa, due to the dominance of animal fats and partially

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hydrogenated vegetable oils in these countries. Enrichment of n-3PUFA in eggs of laying hens is a successful strategy to ensure an adequate supply of n-3PUFA for the greater population. Production of such eggs can be realized by adding common sources of n-3PUFA (i.e., fish oil, marine algae, or flaxseed) to the layer diet (Baucells et al., 2000). The inclusion of n-3PUFA into yolk lipids is feasible and could be achieved by feeding diets rich in n-3PUFA to the laying hens. Fish oils are common feed ingredients used to increase yolk n-3 PUFA in layers (Gonzalez Esquerra and Leeson, 2000). The optimal concentration of n-3PUFA in a layer diet must be optimized experimentally for each production (Pappas et al., 2005). Several studies indicated that dietary supplemented with fish oil had only minor effects on layer performance. Egg production did not affected by including fish oil in commercial layer diets (Schreiner et al., 2004). However others said that egg weight was reduced due to fish oil supplementation (Van Elswyk et al., 1995). Contrarily, Schreiner et al. (2004) indicated that fish oil supplementation had no significantly effect on egg weight and feed intake. Therefore, the objectives of the current study are to produce omega-3 enriched eggs using different quantities of fish oil (0, 1.25, 2.5, 3.5 or 5%) to determine the effects of n-3 PUFA on hens' performance, egg quality characteristics, and egg yolk cholesterol, and quantify alpha-linolenic n-3PUFA, and linoleic n-6PUFA in eggs' yolk from laying hens.

Materials and Methods

The experiment was carried out at the Poultry Research Farm, Department of Poultry Production, Faculty of Agriculture, Kafr El- Sheikh University, Egypt.

Birds and management

One hundred twenty Bovans white hens at their 23rd week of age were housed individually in cages in an open sided house. The birds were kept under a 16 hr light: 8 hr dark lighting schedule (lights on between 06:00 and 22:00 hr). Birds were randomly divided into five dietary treatments and 3 replicates in each (8 birds per replicate); the first treatment group was given 5% fish oil, the second treatment given 3.5% fish oil +1.5% vegetable oil, the third treatment given 2.5% fish oil + 2.5% vegetable oil, the fourth treatment given1.25% fish oil + 3.75% vegetable oil and the fifth treatment given 5% vegetable oil. The ingredient composition and calculated analysis of the experimental diets shows in Table 1. The calculated values of the diets were based on metabolizable energy (ME) values of feed ingredients as listed by national research council (NRC, 2003).

Table 1. Composition of experimental diets.

Ingredient	%				
	Control	T1	T2	T3	T4
Corn	58.67	58.67	58.67	58.67	58.67
Soybean	18	18	18	18	18
Layer concentrate*	10	10	10	10	10
Limestone	7.5	7.5	7.5	7.5	7.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
NaCl	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.03	0.03	0.03	0.03	0.03
Fish oil	0	1.25	2.5	3.5	5
Vegetable oil	5	3.75	2.5	1.5	0
Fatty acids analysis					
N-3 FA[1]	1.01	1.35	2.63	3.76	5.52
SFA[2]	32.02	30.67	29.36	27.1	21.01
USFA[3]	67.98	69.33	70.64	72.9	78.98
Calculated analysis[4]					
CP%	18	18	18	18	18
ME, kcal/kg	2840	2840	2840	2840	2840
Calcium,%	3.8	3.8	3.8	3.8	3.8
Available phosphorus,%	0.44	0.44	0.44	0.44	0.44

N-3 FA means Omega-3 fatty acids; 2SAF means Saturated fatty acids; 3USAF means Unsaturated fatty acids.

4According to NRC (2003); * Layer concentrate provide per kilogram of diet containing the following: 50% crud protein, M.E 2400 Kcal, 3% Available phosphorus, 7.15% Calcium, 3% Lysine, 1.8 Methionine, Vit. A 1.2m IU ,Vit. D3 2.5m IU, Vit. B6 25mg, Vit. B1 10 mg and Vit. B2 50 mg.

Sampling

Egg production was recorded daily; feed consumption, body weight and egg weight were recorded weekly, fatty acids analysis, cholesterol in egg and egg quality was determined every 3 weeks.

Live body weight

The birds were weighed at the start of the experiment, and then they were weighed weekly.

Percentage of egg production

Eggs were collected and recorded daily. Egg production was expressed as hen-day production and calculated using the following equation:

$$\text{Egg production} = \frac{\text{Number of eggs produced}}{\text{Number of live hens housed}} \times 100$$

Feed consumption

The diets were provided regularly at 8 Am daily with enough per weighted ration per each hen. Feed intake per each hen was calculated by figuring out the difference between the weights of offered feed and the remained part at the end of week.

Egg weight

Eggs were collected and evaluated daily.

Egg quality analysis

Egg shape index

The two axis of the egg was measured using the Vernier calipers then calculating egg-shape index as:

$$\text{Egg shape index} = \frac{\text{Transversal axis}}{\text{Longitudinal axis}} \times 100$$

Albumen and yolk height

The height of thick albumen and yolk were measured with tripod micrometer (in mm).

Albumen index and yolk index

Longitudinal and diagonal width of albumen and yolk widths were taken. The albumen and yolk indices were then calculated as follows:

$$\text{Albumen Index} = \frac{\text{Albumen height}}{\text{Albumen width}} \times 100$$

$$\text{Yolk Index} = \frac{\text{Yolk height}}{\text{Yolk width}} \times 100$$

Shell thickness

Shell were rinsed with tap water; the shell membranes manually removed and then dried at 100°C for 2 hr. Shell thickness was measured to the nearest micron by using a metric micrometer equipped with a spherical tip. Estimates were done at the middle part of each egg.

Absolute and relative weight of egg components

Egg was broken and yolk was carefully separated as perfectly as possible by hand and weighed to the nearest gram. The shell was carefully washed to remove the albumen and dried at 100°C for two hours and weighed before cooling (Garlich et al., 1975). Shell weight percentage including the membranes was calculated (shell weight x100/ egg weight). Albumen weight was determined by subtracting yolk weight and dry shell weight from the initial whole egg weight. Relative weight of each egg component to the intact egg weight was then calculated.

Albumen condition (Haugh Units)

This was measured according to the next formula:

Hugh Units= 100x log (H- 1.7 W0.37 +7.57). (1.7, 7.57 and 0.37 are constants).

Where:

H= the height of thick albumen.

W= the egg weight.

Biochemical analysis

Five samples per replicate used for cholesterol and fatty acids analysis. Egg yolk cholesterol was determined by method of Richmond (1973), by using "Cholesterol CHOD-PAP Kits" which produced by Human, Germany. Fatty acids were analyzed by using the method described by Radwan (1978) Lipids were extracted from egg yolk by a mixture of chloroform methanol (2:1) at the ratio of (1:5) in separator funnel and shake carefully for one hour. Let the extract to be separated, the organic layer was taken, then passed through glass funnel contained anhydrous sodium sulfate and finally evaporated to near dryness by a stream of nitrogen Preparation of fatty acids methyl esters from lipids of sample was performed according to the procedure of Radwan (1978). Sample of total lipids (50 mg) was transferred into screw-cap vial, 2ml benzene and 10 ml 1%H_2SO_4 in absolute methanol was added. The vial was covered under a stream of nitrogen before heating in an oven at 90°C for 90 min. Ten ml of distilled water were added to the cooled vial and the methyl esters in each vial were extracted. Ether extracts were combined and concentrated to its minimum volume by using a stream of nitrogen. Analysis of fatty acids was carried out by gas liquid chromatography (GLC) using Shimadzu gas chromatograph GC-4 CM

(PFE) equipped with flame ionization detector (FID) under the following conditions. An analytical glass column (3m X 3mm I'd) packed with 5% (DEGS) diethyl glycol succinct on 80/100 Chrome Operating temperatures (C) for column 180 °C isothermal, injector and detector 270° C, gas flow rates (ml/min) nitrogen 30, hydrogen 1, air 0.5, chart speed 0.5 mm/min. A standard mixture of methyl esters was analyzed under identical conditions prior to running the samples. The retention times of the unknown sample of methyl esters were compared with those of the standard. The concentration of methyl esters were calculated by the triangulation method (Brandt, 1968).

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Statistical analysis

The differences among treatment groups and the control group were analyzed by General Liner model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). The significant differences among means of treatments were compared by Tukey test. $P \leq 0.05$ was set as limit of significance.

Results

Effects of fish oil on final body weight, feed intake, egg weight and egg production are summarized in Table 2. Fish oil increased the final body weight significantly ($P < 0.05$) when the level was 3.5% and 2.5%, respectively, but not in other treatment groups. Feed intake decreased significantly in all experimental groups. Egg weight was decreased ($P < 0.05$) in the treatment groups except 1.25% fish oil. Egg production was decreased by feeding fish oil.

Effects of fish oil on egg quality characteristic are summarized in Table 3. Egg index, albumen height, albumen index, yolk index, haugh units, albumen %, and yolk % were not influenced by feeding fish oil. Shell thickness of eggs from hens fed diets 50 g fish oil/kg diet was not significantly different from those of the control. By feeding fish oil yolk yellow color was increased significantly.

Effects of fish oil on yolk cholesterol, omega-3 and omega-6 fatty acids are shown in Fig.1A,B and C. Yolk cholesterol was decreased significantly in all experimental groups except 1.25% fish oil. Omega-3 fatty acids content in yolk was increased significantly by feeding fish oil while, omega-6 was decreased.

Table. 2. Effect of dietary fish oil on body weight, feed intake, egg weight and egg production.

	Fish oil %				
	Control	1.25	2.5	3.5	5
Final Body weight, g	1321± 39^{b}	1369±41^{b}	1425± 63^{a}	1455±66^{a}	1398±64^{ab}
Feed intake, g/d	121 ± 9^{a}	99± 7^{b}	98± 5^{b}	91± 4^{b}	89± 4^{b}
Egg weight, g	55± 2.1^{a}	54.2± 2^{a}	50.9±1.8^{b}	51.0± 1.8^{b}	50.2± 2^{b}
Egg production, %	89.3± 3^{a}	86± 3^{b}	85.9± 4^{b}	85.2± 5^{b}	88± 3 ^{ab}

Values are expressed as means ±standard error. ; a-b Means with different superscripts differ from each other significantly

Table 3. Effect of feeding different levels of fish oil on egg quality over all mean of experimental.

Characteristic	Fish oil %				
	Control	1.25	2.5	3.5	5
Egg index	77.84±14.11	75.91±2.34	73.93±2.34	72.97±2.23	71.2±2.26
Shell (%)	9.54 ±0.52 ^{b}	9.64 ±0.67 ^{ab}	9.71 ±0.90 ^{ab}	10.04 ±0.58 ^{a}	9.99 ±0.62 ^{a}
Shell thickness (mm)	0.389 ^{b} ±0.03	0.401 ^{b} ±0.031	0.409 ^{b} ±0.022	0.420^{a} ±0.029	0.418 ^{ab} ±0.025
Albumen height (mm)	9.29±1.5	9.39±1.59	9.69±1.06	9.68±1.06	9.6±1.26
Albumen index (%)	13.76±1.96	13.7±2.53	14.36±1.49	14.34±1.4	13.94±1.99
Albumen (%)	65.5±2.71	65.33±2.65	66.17±1.8	66.9±2.18	66.19±1.96
Yolk height (mm)	19.02 ±2.56 ^{a}	18.36 ±1.09 ^{a}	18.53 ±1.15 ^{a}	18.98 ±0.94 ^{a}	16.74 ±0.89 ^{b}
Yolk color	8.12^{b} ±0.81	8.91 ^{a} ±0.77	8.87 ^{a} ±0.74	8.87 ^{a} ±0.61	9.16 ±0.56 ^{a}
Yolk index (%)	45.43±8.88	48.21±4.99	48.72±5.11	48.78±4.26	48.71±3.94
Yolk (%)	23.41±2.81	23.74±2.7	24.11±1.56	24.12±2.14	25.16±1.96
Haugh Units	96.66±7.88	97.45±6.96	98.54±4.66	98.2±4.45	97.66±6.14

Values are expressed as means ±standard error. ; a-b Means with different superscripts differ from each other significantly.

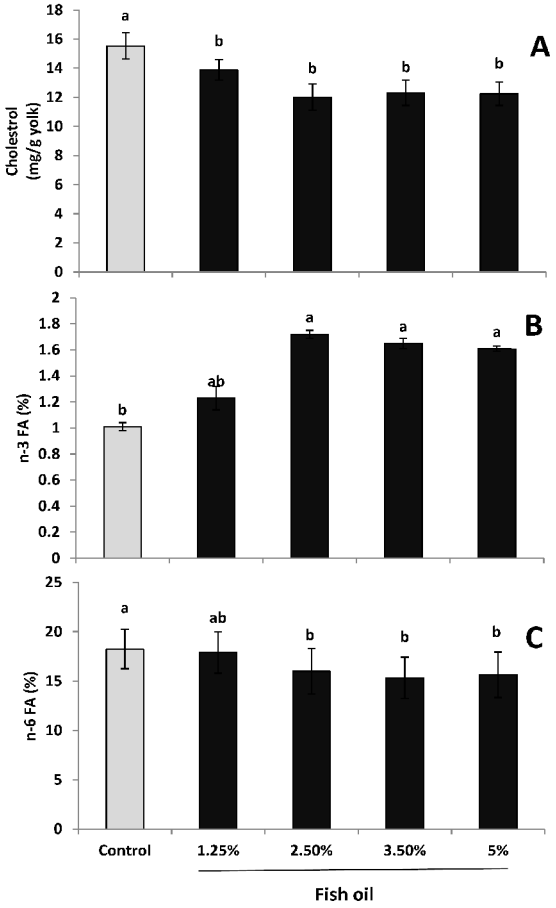

Figure 1. Effect of dietary fish oil on yolk cholesterol (A), omega-3 fatty acids (B) and omega-6 fatty acids (C).
Values are expressed as means ±standard error. ; a-c Means with different superscripts differ from each other (P <0·05).

Discussion

The present study shows that feeding fish oil improved hen's performance due to increased body weight and decreased feed intake. Similar results were documented by Novak and Scheideler (2001). They indicated that a decrease in feed consumption resulted in an increased in hen weights. Generally, previous studies noted that there was no specific trend of the effect of FO on layers body weight. Kang et al. (2001) stated a significant increase in body weight by using cod liver oil. Contrarily, Ahn et al. (1999) found that when laying hen were fed diets containing 0, 2.5, or 5% conjugated linoleic acids, body weight gain was adversely affected by feeding 5% conjugated linoleic acid. On the other hand, Gonzalez and Leeson (2000) proved that when laying hens were fed diets with 2, 4, or 6% menhaden oil; body weight gain from 19 to 55 wk of age was not affected by the dietary treatments. Also, Mostafa et al. (2013) reported that rat's body weight not affected by feeding different source of oils content omega-3. Deborah et al. (2005) showed that when laying hens were fed diets with 0 (control), 1.25, 2.5, or 5% seal blubber oil, the body weight of hens was homogeneous. No significant differences were obtained between control diet and 5.0% FO treatment, meanwhile there was a significant different between control and 1.25, 2.5, and 3.5 % FO treatments. Dietary fish oil has been reported to decrease egg production. This result could be attributed to reduction in feed consumption. Ayerze (1999) reported that the significant differences between treatments in egg production could be due to the somewhat unbalanced test diet, in terms of oil contents. Jones et al. (2000) proved that feed consumption is positively related to egg production; therefore decreasing feed intake will lead to reduce egg production. Similarly, Pal et al. (2004) elucidated that hens fed cod liver oil diets showed the lowest rate of egg production. Also, Shang et al. (2004) observed that when laying hen were fed diets containing 0, 1, 2, 3, 4, 5, or 6% conjugated linoleic acid, egg production was decreased linearly. On the other hand, Basmacoglu et al. (2003) proved that when laying hens were fed different diets containing no fish oil and flaxseed; 1.5% FO; 4.32% flaxseed; 1.5% FO+4.32% flaxseed and 8.64% flaxseed, egg production of hens fed a diet containing 4.32% FO was significantly higher than the controls. The type of diet did not affect egg production traits. Cachaldora et al. (2005) indicated that when hens were fed diets with 2 levels of fish oil (0 and 17 g/kg), egg production characteristics were not affected. These data are in agreement with results found by Carrillo et al. (2005) who proved that when laying hens were fed diets with red crab meal (a source of omega-3 fatty acids), egg production was not influenced by dietary treatment.

Egg quality characteristics were affected by feeding fish oil. Shell thickness, shell percentage, yolk height and yolk color were significantly affected by FO supplementation. Heavier and thicker shells were found in eggs laid in 3.5% FO group. Shell thickness in 3.5% FO group was 0.419 mm while it was 0.389 mm in control group. Similar results were reported by Shang et al. (2004) who proved that dietary conjugated linoleic acid (0, 1, 2, 3, 4, 5, or 6%) increased shells weight and improved shell thickness. On the other hand, Pappas et al. (2005) noted that dietary FO reduced shell thickness compared with dietary soybean oil.

Hammershoj (1995) reported that feeding 3% fish oil resulted in lower shell quality. Novak and Scheideler (2001) postulated that when flaxseed-fed hens produced eggs with less percentage of wet shell than controls. The present study postulated that darker yolk color was obtained in 5% FO treatment. Hammershoj and Parkhurst (l995) noted that yolk color also benefited from omega-3, the oxycarotenoids contained in the diet are lipid soluble and therefore, their absorption from the intestine is likely to be improved when fed the lipids. In fact, yolk color is an important quality trait in influencing consumer acceptance. With respect to the effect of fish oil supplementation on yolk color, Jiang et al. (1992) stated that feeding flax seed to laying hens resulted in darker yolks than other dietary groups, darker yolks might be more appealing to consumers of certain ethnic groups. These observations are in coincident with Hammershoj (1995) who proved that when laying hens were fed diets with fish oil and animal fat, darker yolks were observed in fish oil groups. Herber and Van Elswyk (1998) noted that dietary marine micro algal (as a source of omega-3 PUFA) may also be useful in certain geographical regions for enhancing yolk color.

Egg yolk cholesterol was decreased significantly by feeding fish oil. Nestel et al. (1984) observed that reduction of hepatic synthesis as well as increased proximal beta oxidation activity. Kang et al. (2001) noted that reduction in cholesterol by dietary fish oil suggested mainly from the inhibition of hepatic VLDL production. Novak and Scheideler (2001) found that production of an egg with less yolk and a small decrease in yolk solids. The production of an egg with decreased egg yolk may decrease the amount of cholesterol present per egg consumed. The same trends were stated by Vasko et al. (2005). Ouyang et al. (2004) noted that when laying hens were fed with diet supplemented with 5% fish oil, 5% palm oil, and 5% soybean oil, the cholesterol level in yolk of fish oil group was lower than Palm oil and control ($P<0.01$). The results demonstrated that fish oil could decrease the cholesterol. Vasko et al.(2005) found that when laying hens were fed three diets supplemented with flax oil and fish oil, concentrations of cholesterol in the egg yolk significantly decreased in the groups with supplementation of flax and fish oil (11.98 and 11.79 mg/g). Omega-3 fatty acids were increased while omega-6 was decreased by feeding fish oil. Several attempts have been made to reverse the decline in human egg consumption by changing egg composition through feeding hens modified diets. Incorporation of n-3PUFA in egg yolks by changing hen diets has been more successful and has been reported in a number of studies (Caston et al., 1994; Scheideler and Froning, 1996). These experiments altered the yolk fatty acid profile by adding either fish products or flax seed to the hens' feed. Similar findings were detected in the present study. Generally, the fatty acid composition of the yolks reflected the dietary fish oil levels. The changes in yolk omega-3 and omega-6 fatty acids shown here due to feeding graded levels of fish oil are in agreement with previous reports (Van Elswyk et al., 1995; Van Elswyk 1997; Hoffman et al., 2004). They reported that the most significant effect of dietary fish oil was on long chain n-3 PUFA. Moreover, Schreiner et al. (2004) observed that feeding fish oil at 1.25% led to an increase ($P < 0.0001$) in the long-chain n-3 PUFA in the egg yolk lipids. Egg enriched with n-3PUFA may be associated with off-odours and in particular fishy taints. However, these can be minimized if the hens are fed 3.5% or less of high quality fish oil or 5% or less flaxseed (Deborah et al., 2005). Van Elswyk et al. (1994) indicated that egg yolk n-3 fatty acids content was significantly increased when hens fed diets enriched with 3% fish oil. Kang et al. (2001) observed that when hens were fed on a modified diet in which 3% cotton seed oil was replaced by active EPA-cod liver oil, an increased in Linolenic acid was detected.

In conclusion

The present study clearly shows that by add 3.5% of fish oil in laying hens diets, egg cholesterol can be decreased this due to reduction of hepatic synthesis as well as increased proximal beta oxidation activity or due to the inhibition of hepatic VLDL and LDL production. Also, egg quality was improved by increased in shell thickness and yolk color. Furthermore, add 3.5% fish oil in the diets can change the fatty acids profile in the liver then these fatty acids were enriched in yolk. So in future we can produce a health eggs by feeding fish oil to laying hens and human consuming this type of eggs may have beneficial health effect.

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ANIMAL SCIENCE

Comparison of sample preparation for the isolation of *Listeria* species in naturally contaminated catfish and tilapia samples

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Abstract

In this study, two preparation sample (pelleted and non-pelleted) and two pre-enrichment (with and without pre-enrichment in Half Frazer Broth) were assessed on their efficacy to isolate *Listeria* sp. from naturally contaminated catfish and tilapia samples. A total of 108 samples (32 catfish intestines, 32 tilapia intestines, and 44 water samples) were examined for the presence of *Listeria* sp. Out of 16 *Listeria* sp. were observed on pelleted sample and 5 *Listeria* sp. were observed on non-pelleted sample. All samples were preceded without pre-enrichment. Nineteen (19) isolates were observed on pelleted sample 17 on non- pelleted sample. Those were combined with pre-enrichment. The study revealed that, the isolation of *Listeria* sp. by pelleting the sample in the combination with pre-enrichment from naturally contaminated catfish, tilapia and water samples were relatively higher than their opponents. By improving the efficacy of isolating *Listeria* sp. is beneficial for reporting the presence of these pathogenic bacteria due to public health purposes.

Key words: Catfish, Isolation method, *Listeria*, Pelleted, Tilapia

Introduction

Catfish and tilapia are the highest fresh water fishes reared in the ponds in Malaysia. Based on the recent data, total production of catfish and tilapia were 64.9% and 18.2%, respectively (Fisheries Department Malaysia 2010). However, catfish and tilapia are potential agents of pathogenic bacteria such as *Listeria* sp. (Chen et al., 2010; Jaleewar et al., 2007).

Listeria sp. is a gram positive and grows in a wide temperature range, from -1.5 to 45ºC (Adam and Moss, 2004). The growth of the organism at -1.5ºC was very slow, with a lag time of 174 h (Hudson et al., 1994). *Listeria* could move with flagella and polymerizing actin comet tails with a protein called ActA. Some studies suggested that 1 to 10% of humans may carry *L. monocytogenes* in their intestines (EMLab, 2009).

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Listeriosis, caused by *L. monocytogenes*, is a food borne infection of great public health concern due to its clinical severity and high fatality. Mostly affected by severe disease are people who are elderly or immunocompromised, pregnant women and neonates (younger than four weeks). *L. monocytogenes* could cause meningoencephalitis and septicemia in newborns, elderly, immunocompromised patients and abortion in pregnant women (Marchant, 2003). The infective dose of *L. monocytogenes* is unknown but it is believed to vary with the strains and susceptibility of the victim. Some studies has reported to find the antibacterial to against this pathogenic bacteria (Mattana et al., 2012; Satorres et al., 2012, 2013).

The conventional isolation method of Listeria sp. was established by ISO 11290 (ISO 2004; ISO, 1998; ISO, 1996). However, there is limited data regarding the isolation method of *Listeria* species by pelleted sample to increase the yield Thus, this study was carried out to fill this gap. The aims of this study were to compare on two preparation sample (pelleted and non-pelleted) and two pre-enrichment (with and without pre-enrichment in Half Frazer Broth) and to determine their efficacy on *Listeria* isolation from naturally contaminated catfish and tilapia samples.

Material and Methods

Samples

The microbial quality of the catfish and tilapia were investigated by taking samples of intestines. The fish were taken from local wet markets and ponds on November 2008 to September 2009. The fishes were sampled from each wet market and pond in Penang and surrounding Penang Malaysia. The samples were placed in sterile bags. Water samples were collected in sterile test tubes, covered, and transferred to the laboratory within minutes.

Isolation of *Listeria* sp.

In method A (pellet method), the intestines of fish were removed using a sterile knife and were pooled by using sterile forceps. The intestines were placed on a sterile tray wrapped in aluminum foil and chopped thoroughly with sterile knife. Twenty five grams of the intestines were placed in a stomacher bag containing 225 mL 0.1% Peptone Water (PW, Oxoid, Basingtoke, Hampshire, United Kingdom) and homogenized using a stomacher (Interscience, France) for 2 min. The homogenate was divided equally, placed in 50 mL centrifuge tubes and centrifuged for 15 min at 10,000 x g to obtain a pellet. The pellet was pre-enriched by re-suspending it in 10 mL Half Frazer Broth (HFB, Merck KGaA, Darmstadt, Germany) and incubated at 37^{o}C for 24 h. In method B (non-pelleted method), 25 g of chopped intestines were placed in a stomacher bag and pre-enriched by homogenizing with 225 mL HFB using a stomacher for 2 min and incubated at 37^{o}C for 24 h. Twenty five g of intestines were also directly pre-enriched by homogenizing in 225 mL of HFB, using a stomacher for 2 min and incubated at 37^{o}C for 24 h. After pre-enrichment, 1 mL portions were transferred into 10 mL of Frazer Broth (FB, Merck KGaA, Darmstadt, Germany) and incubated at 37^{o}C for 24 h. Following enrichment, 10 µL of the culture was streak-plated on to ALOA (Merck KGaA, Darmstadt, Germany) and PALCAM (Merck KGaA, Darmstadt, Germany) and were incubated at 37^{o}C for 24-48 h.

Twenty five mL of water samples were pelleted by centrifuging at 10,000 x g for 15 min, the pellet was re-suspended and pre-enriched in 10 mL of HFB and incubated at 37^{o}C for 24 h. Twenty five mL of water samples were also directly pre-enriched by homogenizing in 225 mL of HFB, using a stomacher for 2 min and incubated at 37^{o}C for 24 h. After pre-enrichment, 1 mL of both pelleted and non-pelleted methods was enriched in 10 mL of FB and incubated at 42^{o}C for 24 h. After enrichment, 10 µL of culture was streak-plated onto ALOA (Merck KGaA, Darmstadt, Germany) and PALCAM (Merck KGaA, Darmstadt, Germany) and were incubated at 37^{o}C for 24-48 h. The protocol was based on the modification of EN ISO 11 290-1. Presumptive *Listeria* colonies were picked, purified Gram stained and subjected to following biochemical tests; gram staining, catalase, cytochrome oxidase, microscopic observation, motility test and haemolysis test. Microbact *Listeria* Identification System 12L (Oxoid, Basingtoke, Hampshire, United Kingdom) was used to identify *Listeria* sp.

Preparation of genomic DNA

Single colony of pure *Listeria* culture was inoculated into 5 mL Triptic Soya Broth (TSB, Merck KGaA, Darmstadt, Germany) and incubated in orbital shaker (with vigorous shaking) at 37°C for 16 to 18 h. The overnight culture (5 mL) was centrifuged for 5 min at 1000×g to obtain pellets. Pellets were dried and subjected to plasmid DNA extraction and purification using Wizard Genomic Purification Kit (Promega, Madison, USA) by following the manufacturer's instructions (Anonymous, 2012).

Detection iap gene of *Listeria* species by PCR

The PCR was set for 25 µl reaction volume. Initially, for the detection of individual *iap* gene of *Listeria* sp., PCR conditions were used with modification method of Chen and Knabel (2007). The reaction mixture for PCR was optimized as follows; 10× PCR buffer (Sigma), 0.2 mM dNTP mix (Promega), 2 mM $MgCl_2$ (Promega) and 0.1 µmol of forward and reverse primer of each set, 1.6 unit of Taq DNA Polymerase (Promega), 2.5 µl of cell lysate and sterilized water free nuclease (Promega) to make up the reaction volume. The primers were used according to Bubert (1992); IAPF (5'- ATG AAT ATG AAA AAA GCAAC-3') and IAPR (5'-TTA TAC GCG ACC GAA GCC AAC-3'). Temperature cycling was performed according to Chen and Knabel (2007) using a thermocycler (Biometra, Germany) with an initial activation step for 5 min at 95°C prior to 15 cycles of 1 min at 94°C, 1 min with a touchdown from 55°C to 51°C (3 cycles per temperature), and 1 min at 72°C, followed by 15 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C and 1 final cycle for 8 min at 72°C. The PCR products (10 µl reaction mixture mixed with 2 µl loading dye) were separated by electrophoresis on a 1.5% agarose gel (Promega, USA) and was run at 90V for 1h 20 min. Ladder 100 bp (Promega, USA) was used for molecular weight marker.

Statistical analysis

The General Linear Model Procedure (SPSS version 13, USA) at the significance level ($P<0.05$) was used to assess differences of pellet and non-pellet.

Results and Discussion

Listeria species are well known pathogenic bacteria in most food-borne diseases which cause the highest mortality rate. Symptoms of *Listeria* infection are gastroenteritis, meningitis, abortion, and death in some cases (Adams and Moss, 2004). Thus, the methods which ensure the highest possible recovery of these pathogens are important. In this present study, centrifuging sample to obtain the pellet was shown to be relatively higher to isolate *Listeria* species. The material in the suspension was against the walls and separated from the solution by centrifugal force. The samples which may be contaminated by *Listeria* can be more concentrated in to the pellet form. Thus, the number and chance to isolate *Listeria* might be increased.

This study found that pelleted method with pre-enrichment was significant ($P<0.05$) with non-pelleted method on *Listeria* isolation (Table 1). The highest yield using pelleted method in fish and water were 8/64 and 11/44. The yields obtained from pelleted method were relatively higher than those using non-pelleted method. The sensitivity was relatively higher on pelleted method compared to non-pelleted method. The sensitivity of pelleted method in combination with pre-enrichment was 1 and 0.92 for fish and water samples. The sensitivity of non-pelleted method in combination with pre-enrichment was 1 and 0.75 for fish and water samples. Even though the centrifuged bacteria has been reported to be damaged on cell membrane (Bell, 2005), the bacteria might be repaired in enrichment broth with or without pre-enrichment step.

The sensitivity of pelleted method without pre-enrichment in HFB was found to be high for both fish (1) and water (0.75) samples. The pelleted method with pre-enrichment in HFB yielded relatively high for fish (1) and water (0.92) samples. HFP has a high buffering capacity which may repair the injured Listeria (Holbrook et al., 1992). HFB contained lithium, acriflavin and nalidixic acid (Frazer and Sperber, 1988). The Half Frazer Broth, which contained of half concentration of acriflavin and antibiotics, was aimed to allow the better growth of injured *Listeria* (Holbrook et al., 1992).

This present study isolation of *Listeria* without pre-enrichment step was observed (Table 1). The enrichment for *Listeria* use FB which was effective for the growth of *Listeria* sp. and the growth of enterococci was inhibited (Frazer and Sperber, 1988). Thus, the combination of pre-enrichment and enrichment could increase the growth of *Listeria* species.

The presence of *Listeria* sp. was detected by PCR to observe iap gene. The genus *Listeria* sp. contained the genetic marker such as iap gene which encoded a major extracellular protein p60 (Bubert et al., 1992). In this present study, this gene was observed on all *Listeria* sp. isolates. The representative gel was shown in Figure 1.

Table 1. Positive sample of *Listeria* sp. using two preparation sample (pelleted and non-pelleted sample) and two pre-enrichment (with and without pre-enrichment in Half Frazer Broth).

Sample	Method	Pelleted method		Non-pelleted method	
		Total positive	Sensitivity[c]	Total positive	Sensitivity
Fish	Without Pre-enrichment (*n*=64)	6[b]	0.75	1[a]	0.13
	With Pre-enrichment (*n*=64)	8[a]	1	8[a]	1
	Total positive	8		8	
Water	Without Pre-enrichment (*n*=44)	10[b]	0.83	4[a]	0.33
	With Pre-enrichment (*n*=44)	11[a]	0.92	9[a]	0.75
	Total positive	12		12	

a,b = different alphabet means significant different at $P<0.05$ in the same row; c = sensitivity is calculated in relation to the total number of positive sample

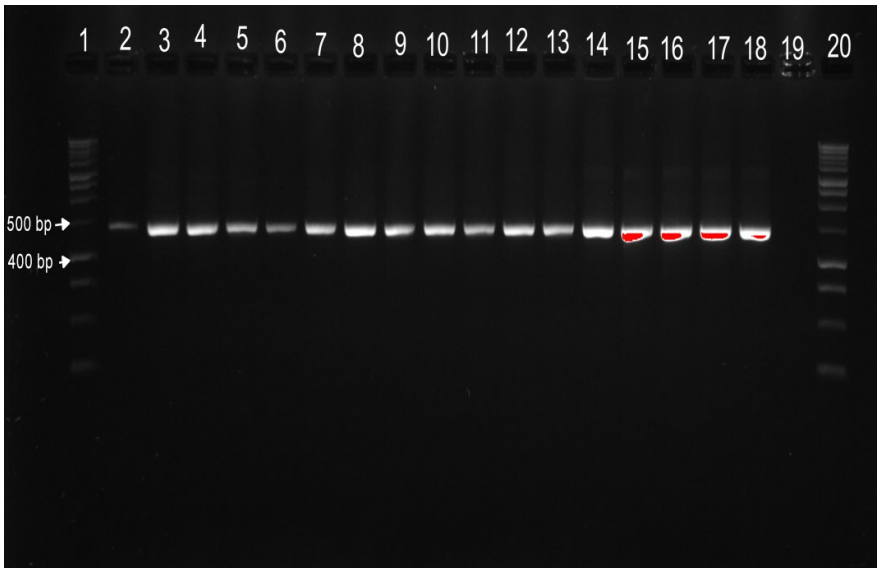

Figure 1. The representative gel of iap gene in *Listeria* isolates isolated from catfish, tilapia and water.
Lane 1,20: Marker 100 bp; Lane 2 : positive control L. monocytogenes; Lane 3-18 : samples; Lane 19 : negative control

The prevalence of *Listeria* sp. in catfish and tilapia were 3/32 and 5/32. The prevalence of *Listeria* sp. in tilapia and its water was relatively higher than catfish and its water (Table 1). Other study found that *Listeria* was found in tilapia and catfish (Chen et al., 2010; Jaleewar et al, 2007). This study found 3 isolates of *L. ivanovii* isolated from catfish, 2 isolates of *L. ivanovii*, 2 isolates of *L. grayi* and 1 isolates of *L. welshimeri* isolated from tilapia. The prevalence of *Listeria* sp. in water obtained from catfish tank and ponds were 5/32. These were 4 isolates of *L. ivanovii* and 1 isolates of *L. welshimeri*. The prevalence of Listeria spp. obtained from tilapia pond were 7/12 which were 3 isolates of *L. ivanovii*, 2 isolates of L. grayi, 1 isolates of *L. welshimeri* and 1 isolates of *L. welshimeri*.

L. ivanovii was the predominant in tilapia, catfish and water. Chen et al. (2010) found that *L. ivanovii* and *L. monocytogenes* were observed in catfish. The important finding of this study was the presence of *L. ivanovii* and *L. monocytogenes*. Guillet et al. (2010) revealed that *L. ivanovii* and *L. monocytogenes* could make human disease.

Conclusion

Pelleted method can be the new alternative to isolate *Listeria* species. This new method can be combined with and without pre-enrichment to isolate *Listeria* sp. Pelleting sample in combination with pre-enrichment yielded higher sensitivity compared to non-pelleting sample and other. The presence of *L. monocytogenes* and *L. ivanovii* in catfish and tilapia become food safety concern for the public health.

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ANIMAL SCIENCE

Seasonal and physiological variation of gross composition of camel milk in Saudi Arabia

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Abstract

Weekly milk samples from ten lactating she camels (*Camelus dromedarius*) were analyzed regularly for 11 months after parturition. The main values for all samples were 2.54 ± 0.72g/100g fat matter, 3.07 ± 0.30g/100g protein, 4.21 ± 0.37g/100g lactose and 0.76 ± 0.10g/100g ash. Fat content decreased from 3.41% at the first week to 2.29% at 36th week post-partum with rising at the end to 2.95% while protein decreased from 3.44% at week 1 to 2.79% at the end of lactation, and lactose from 4.48% to 3.90%. Ash increased from 0.72% to 0.82% then decreased down to 0.71%. Regarding seasonal variation, maximum level of fat was observed in January (3.46%) and minimum at summer time (2.29% in July). Protein content was maximum in February (3.32%) and minimum in October (2.76%). For lactose, the maximum mean value was 4.38% in February and the minimum in September (3.83%). The ash content was quite variable in January then stable all over the year. All components were highly positively correlated, except between fat and ash content which was not significant. No significant effect of parity, gestation length, calf body weight at birth or adult weight on all milk content. The average total milk production was 1207 L for 11 months range between 875 and 1616 L. The correlation between milk production and milk components are significantly negative.

Key words: Camel milk, Milk components, Seasonal variation, Physiological stage, Milk yield

Introduction

The camel milk has a good nutritive quality and can be a convenient source of nutrient in human diet in arid and semi-arid zones. A lot of information is still to be generated about camel milk as a source of food (Igbal and Younas, 2001). Camel milk is a complex mixture of fat, protein, lactose, minerals, and vitamins and miscellaneous constituents dispersed in water. Wide variation in constituents of camel milk is attributed to some factors such as parity, season and physiological stage. The data of physiological change of the main components of camel milk are very scarce (Konuspayeva et al., 2009a) and generally based on monthly reported data. In the present study, data on week basis were regularly reported. Thus, the objectives of the study were (i) to determine in camel milk, the individual chemical composition (fat, protein, lactose and ash), (ii) to assess the changes during lactation and season, and (iii) to study the relationships between milk yield and milk constituents.

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Material and Methods

Location and animals

This study was carried out in Al -Jouf 'Camel & Range Research Center' located in north-west Saudi Arabia, 950 km from Riyadh. Average annual temperature was 20°C, ranging from 12°C to 27°C, and average annual rainfall were 55 mm. The herd was composed by camels of four ecotype breeds (Malhah, Wadhah, Hamrah and Safrah) and their crosses (Abdallah and Faye, 2012). Camels were kept indoor throughout the year and housed in pens. Their diet was composed of alfalfa, barley, and salt and wheat bran.

For each animal, the following parameters were reported: parity (primiparous or multiparous), gestation length, calf weight at birth and adult weight at calving date. The quantitative data were shared into 2 modalities for statistical analysis (Table 1).

Table 1. Modalities of the explaining factors of the milk components in ANOVA model.

Factors	Modalities	values	Mean
Gestation length	Short	< 389 days	378 days
	Long	> 390 days	395 days
Calf weight	Low	<39 kg	36 kg
	High	>40 kg	43 kg
Adult weight	Low	<600 kg	575 kg
	High	>601 kg	700 kg
Parity	Primiparous	1	1
	Multiparous	2 and more	5.1
Breed	Safrah		
	Waddah		
	Homor		
	Majaheem		

Milk sampling

Milk samples were collected early morning at milking time (6:00 in winter and 5:00 in summer) in clean plastic bottles (40 ml). After thoroughly mixing for getting homogeneous sample, they were immediately transferred to laboratory close to the farm for analysis at the temperature of air-conditioned room. The time between sampling and analysis did not exceed one hour. All the samples came from 10 she-camels with different breeds, parity, gestation length, weight, calf body weight and age. The milk sampling was achieved every week at the same day in order to get a strictly 7-d interval.

Milk analysis

Chemical component of milk percentage of fat, total protein, lactose and ash was determined weekly for 10 months after parturition by automatic milk analyzer device (lactoscan MCC) calibrated for camel milk. Density and conductivity were also reported. The ultrasonic technology used by Lactoscan allowed direct measurement of fat, proteins, lactose and salts (see http://www.lactoscan.com/products_MCC.html). Lactoscan determined also the freezing point of each sample and the quantity of added water. The freezing point was calculated automatically from the components it depends on. The measuring range and the accuracy of the apparatus are reported in Table 2.

Statistical analysis

Physiological and seasonal changes (mean and SD) were studied at weekly and /or monthly basis. The correlations between the different components of the camel milk were calculated using Pearson correlation. The effects of some parameters were investigated as parity, gestation length, breed and body weight by ANOVA procedure. The differences between modalities reported in table 1 were investigated by Fisher test (LSD).

The data of present study was analyzed by using XLSTAT software (2010, 2.02 version, Addinsoft ©).

Table 2. Measuring range and accuracy of the apparatus Lactoscan MCC for the different milk parameters.

Parameter	Measuring range	Accuracy
Fat	0,01– 45%	±0,06%
Solids-non-fat (SNF)	3% – 40%	±0,15%
Density	1000 – 1160 kg/m^3	±0,3kg/m^3
Protein	2% – 15%	±0,15%
Lactose	0.01% – 20%	±0,2%
Added water	0% – 70%	±3%
Milk sample t°	5°C – 40 °C	±1%
Freezing point	–0.4°C — –0.7°C	±0,005%
Salts	0,4% – 4%	±0,05%
pH	0 – 14	±0,05%
Conductivity	2 – 14 [mS/cm]	±0,05%
Total solids	0 – 50%	±0,17%

Results

The mean values for all samples were 2.54 ± 0.72 g/100g fat matter, 3.07 ± 0.30 g/100 g protein, 4.21 ± 0.37 g/100 g lactose and 0.76 ± 0.10 g/100g ash.

Weekly change of fat, protein, lactose and salt

Regarding the weekly change along the lactation stage, the fat content was regularly decreasing, passing from 3.41% at the first week post-partum to 2.29% at the 36th weeks post-partum, but with a rising at the end of lactation up to 2.95% at the 43th week (Figure 1).

Different patterns were observed for protein, showing a regular decreasing from 3.44 % at week 1 to 2.79 % at the end of lactation, and lactose (from 4.48 to 3.90 % Figure 1).

The ash content was low after parturition (0.72%) with an important increase, up to the lactation peak (2 months after delivery), then a regular decreasing down to similar level of post-partum period (0.71%) (Figure2).

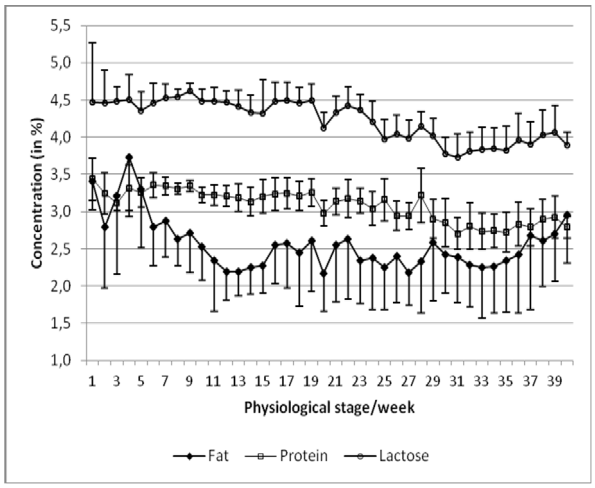

Figure 1. Weekly changes all along the lactation of fat, protein and lactose in camel milk.

Seasonal variation

The fat content decreased regularly all along the year with a maximum level (3.46 %) in January and a minimum at the summer time (2.29% in July, i.e at the warmest month). At autumn, corresponding to colder time and to the end of lactation, the fat content increased again to reach similar value than in February (2.73%). Protein and lactose showed a quite different pattern with a slight increase from January to February, then a high decrease in autumn. The protein content was maximum in February (3.32%) and minimum in October (2.76%). For lactose, the maximum mean value was 4.38% in February and the minimum occurred in September (3.83%) (Figure 3).

The ash content was quite variable in January (very high standard-deviation) and relatively stable all along the year with a slight decrease in autumn (Figure 4).

Correlation between all parameters

All components (fat, protein, lactose and ash) and density were highly positively correlated ($P < 0.0001$) except between fat and ash content and between fat and density which were not significant (table 2). The correlation of each parameter with the conductivity was negatively highly significant ($P < 0.001$) also (Table 3).

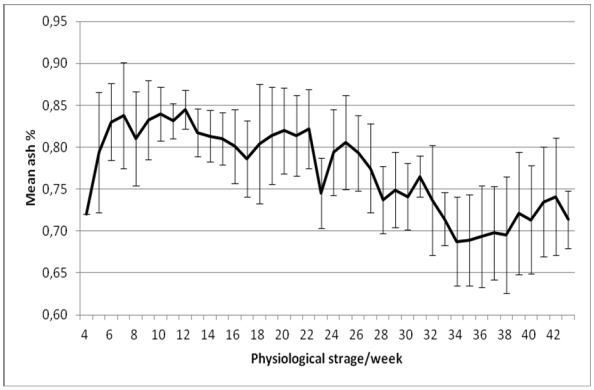

Figure 2. Weekly change after delivery in ash content of camel milk.

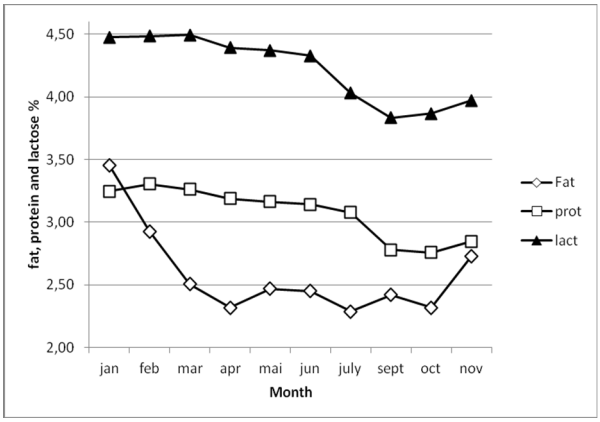

Figure 3. Seasonal variation of fat, protein and lactose content in camel milk.

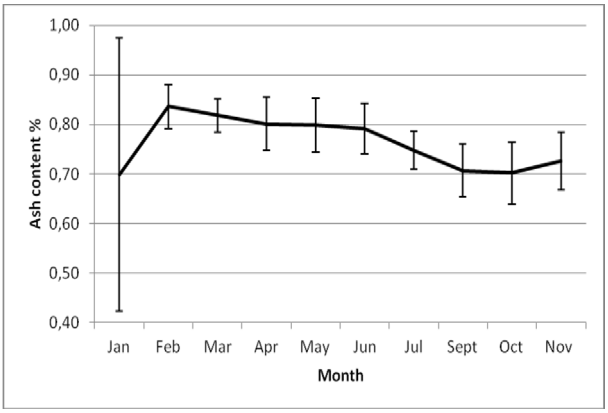

Figure 4. Seasonal variation of salt in camel milk.

Table 3. Matrix of correlation coefficient (r) between chemical and physical parameters of the camel milk (n = 347).

Variables	Fat	Density	Lactose	Ash	Protein	Conductivity
Fat	**1**	0,042	**0,350**	0,205	**0,326**	**-0,371**
Density	0,042	**1**	**0,511**	**0,899**	**0,509**	**-0,406**
Lactose	**0,350**	**0,511**	**1**	**0,579**	**0,916**	**-0,760**
Ash	0,205	**0,899**	**0,579**	**1**	**0,584**	**-0,460**
Protein	**0,326**	**0,509**	**0,916**	**0,584**	**1**	**-0,734**
Conductivity	**-0,371**	**-0,406**	**-0,760**	**-0,460**	**-0,734**	**1**

The values in bold are at P < 0 .05 significant level.

Table 4. Mean and SD of the main components of camel milk (in %) according to parity, breed, calf weight at birth, mother weight and length of lactation (all differences were no significant).

Variation factor	Parameter				
		Fat	Protein	Lactose	Ash
Parity	Primiparous	2.66±0.72	3.22±0.27	4.37±0.32	0.80±0.06
	Multiparous	2.45±0.61	3.11±0.25	4.26±0.34	0.78±0.06
Breed	Homor	2.36±0.58	3.06±0.25	4.18±0.35	0.77±0.06
	Majaheem	2.48±0.72	3.15±0.26	4.33±0.32	0.79±0.22
	Safrah	3.01±0.64	3.23±0.30	4.36±0.36	0.81±0.07
	Waddah	2.36±0.57	3.16±0.36	4.34±0.31	0.79±0.06
Calf weight	High	2.42±0.57	3.07±0.27	4.20±0.34	0.77±0.06
	Low	2.56±0.69	3.18±0.28	4.34±0.33	0.80±0.06
Adult W	High	2.47±0.69	3.15±0.24	4.25±0.33	0.78±0.06
	Low	2.54±0.61	3.18±0.28	4.33±0.35	0.79±0.07
Gestation length	Long	2.49±0.67	3.15±0.27	4.31±0.33	0.78±0.06
	Short	2.54±0.61	3.11±0.26	4.26±0.35	0.79±0.06

Effect of parity and gestation length, calf body weight at birth, adult weight and breed

No significant effect of parity, gestation length calf body weight at birth or adult weight on all parameters in milk was reported (Table 4). The between-camel variability was higher for fat content, the mean individual value varying from 2.00 to 2.98 % according to the camel. The ranges were 2.91 to 3.22 for protein, 4.02 to 4.41 for lactose and only 0.74 to 0.82 % for ash content.

Relationships with dairy yield

The total milk production (not including the part milked by the camel-calf) was on average 1207 L for 11 months of lactation with a range between 875 and 1616 L. The lactation curve on week basis (Figure 5) showed a classical pattern with a lactation peak starting on 13th week with a plateau up to 24th week. The persistence coefficient was high with an ascending period between 160.6 (2nd month/first month of lactation) and 100.6% (6th month/5th month) and a descending period starting at 98.7% (7th month/6th month) to 87% (11th month/10th month).

As usual, the correlation between milk production and concentration of the different milk components were significantly negative (P<0.001). However, the total quantity of exported components in milk was mainly depending of the total milk production (Figure 6). The total quantity of fat varied on average from 54 g at the first month of lactation up to 152 g at the month 6. The minimum and maximum values for protein quantity (54g and 195g) were observed at the same time than fat component. For lactose (74 g and 274g), the maximum was observed at month 5 as for ash quantity (14g and 50 g respectively).

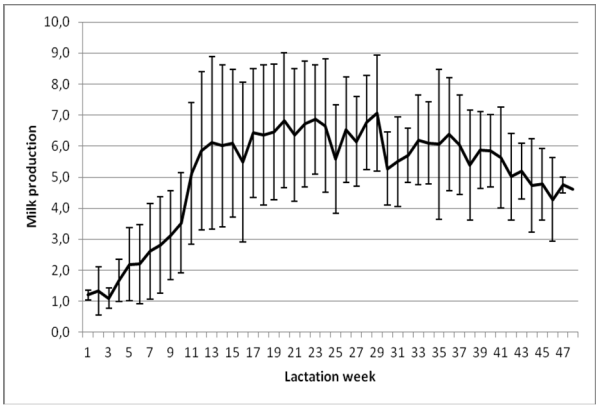

Figure 5. Lactation curve of camel on weekly basis (mean daily production in l).

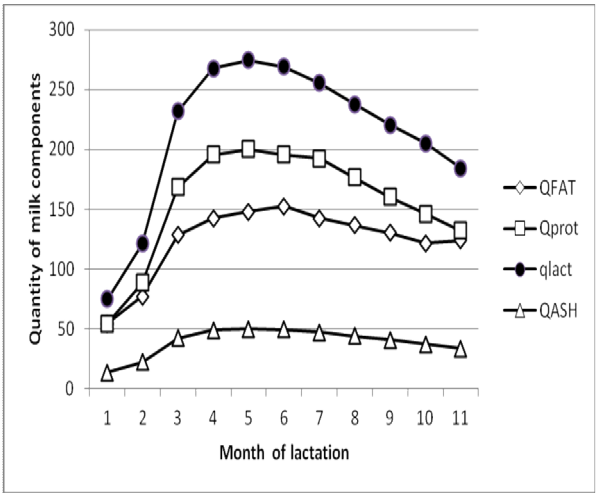

Figure 6. Physiological variation monthly basis of total quantity of fat (Qfat), protein (Qprot), lactose (Qlact) and ash (Qash) exported in camel milk (in g).

Discussion

Milk composition

In general, the present study showed a wide variation in the gross composition of camel milk. The values of the main components observed in our study were in the range of the study of Khaskheli (2005) and Mint Meiloud et al. (2011) except the ash content which was appeared lower than in these two references. The variation in mineral reflects many genetic and environmental factors such as the system of feeding and browsing of different plants. The mean content of fat and protein in our study shows a lower results compared to those reported by Konuspayeva et al. (2009b), Igbal et al. (2001) and Zeleke (2007), but otherwise close to results reported by Farah and Fisher (2004). This wide variation in the milk constituents is generally attributed to some factors such as breed, herd management, sampling technique used and feed quality (Al-Shaikh and Salah, 1994).

Seasonal and physiological variation

As the calving season in camel is generally grouped within 2-3 months, the seasonal variation in milk composition is a combination of physiological stage, feeding system and climatic conditions. In our study, the calving season occurred between December and February. The highest variability was observed for milk fat. Protein and lactose are also affected by season (Bakheit et al., 2008). Shuiep and Elzubeir (2008) and Haddadin et al. (2008) reported a minimum fat content in camel milk at the hot season while it was in autumn for protein and lactose. In our study, the fat percentage dropped to a lower level during the hot months (April to September), this period corresponding in the same time to the maximum of lactation. Similar observations were reported by Igbal (2001). Indeed, the fat component is the most sensitive parameter to the dilution effect linked to the production increase (Firkins and Eastridge, 1992).

The ash content showed quite variable pattern in same line to that reported by Zhang (2005) on Bactrian camel.

The higher concentration of organic milk components at the early stage of lactation was also reported by Zeleke (2007) and was regarded as in higher level than in late stage at the opposite of the results reported by Sahani et al. (1998).

Effect of parity and other animal characteristics

In our study, there was no significant difference between primiparous and multiparous camel contrary to Zeleke (2007) reporting that parity had significant effect on fat, protein and dry matter of camel milk in Eastern Ethiopia. In his study, the highest percentage composition of protein, fat, and dry matter was recorded from camels at the 3rd parity. In our study the number of different parities was not sufficient to detect any significant statistical effect.

El-Mougy (1995) revealed wide variation in milk composition between three breed in Saudi Arabia (Majaheem, Waddah and Hamra) likewise Gaili et al. (2000) reported difference in Saudi Arabia between two Saudi camel breeds namely Majaheem and Waddah. The absence of effect due to parity, breed, gestation length, and adult weight on all parameters in milk may need probably a larger number of animals for analysis.

Effect of milk yield

The mean milk yield reported in our study was closed to data recorded by Salla *et al*, (2010) and Gaili et al. (2000) on similar camel population in Saudi Arabia. Regarding the whole production at lactation level, the mean of 1207 l observed in our farm appeared lower than the values recorded by many authors (for example Aslam et al., 2002). However, based on 5 years monitoring, the average dairy yield reported in the same farm than the present study was 1970 l (Musaad et al., 2013). In the Arabian Emirates, the average milk yield was set around 2000 litres per lactation (Quandil and Oudar, 1984). Sohail (1983) reported that, on average, Arabian camels can produce up to 2 275 litres of milk per year. Shareha (1985) reported in Syria 7.3 to 12.2 litres daily when the udder was completely milked. According to Qureshi (1986), a

camel may produce on average 8 to 20 litres of milk daily, but under intensive management conditions it may produce from 15 to 40 litres daily. In Kuwait, a good, a medium and a poor camel milk producer can produce 9030, 3185 and 805 litres respectively in 350 days (Ibnoaf, 1987). In Saudi Arabia, the average milk yield ranges from 2.4 to 7.6 litres daily (Basmaeil and Bakkar, 1987). El-Naggar (1998) reported that the camel can yield about 2 700 to 3 666 litres per lactation. These differences could be partly attributed to the data collection procedure. Indeed, the estimations of camel milk yield available in the literature mention the quantities produced per lactation or year. In most of the cases, the authors did not specify if the yield included or not the part drunken by the young camel which represents about 40% of the entire production, sometimes even 75% under certain conditions (Faye, 2005). In our study, the part taken by the young camel was not included.

The peak of lactation in our study (third to six months of lactation) did not agree with the observation of Khan and Iqbal (2001) who observed a peak yield during the second to the third month of lactation.

The correlation between milk production and milk components was significantly negative due to the dilution effect, widely described already in other dairy animals.

Conclusion

Camel milk composition showed a wide variability in its constituents depending on the physiological, genetic and environmental factors. However, more studies with larger number of animals and ecotypes must be generated for a better evaluation of the management factors. The contribution of the camel to the world milk supply is marginal but essential for human populations in arid and semi-arid areas. However, available data on the camel's production potential and composition are not sufficient. The great variation in camel milk production may be attributed to the methods employed to determine yield as it has been suggested above. Further investigations and probably standardization of the methods are necessary to point out the importance of camel milk production for the food security of desert areas in the world.

The international scientific community has to turn its attention to a good performance control of dairy production in camels. Specific tools for dairy yield monitoring are necessary.

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ANIMAL SCIENCE

Classification of Maghrebi camels (*Camelus dromedarius*) according to their tribal affiliation and body traits in southern Tunisia

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Abstract

Five groups of Maghrebi camels have been identified in southern Tunisia according to their tribal affiliation: the Ourdhaoui Médenine in Tawazins tribe, the Ourdhaoui Tataouine in Oudarna tribe, the Guiloufi in Beni Guilouf tribe from Kébili, the Gueoudi at Ouled Gharib tribe from Kébili and the Merzougui in the Marazigues tribe from Kébili. The identification of these groups was achieved by recording nine body measurements (length of the body, length of the neck, thoracic girth, abdominal circumference, height at the hump, height at the withers, width between shoulders, length of the anterior limb and the length of the tool) from a total of 304 female camels (age ≥ 6 years). Positive correlation was observed between the height at the hump and height at the withers, the thoracic girth and the abdominal circumference, the length of the body and the length of the neck. However, the length of the tail showed a negative correlation with the abdominal circumference and the height at the withers. Gueoudi, Guiloufi and Merzougui camel groups had the higher body length and the higher abdominal circumference compared to Ourdhaoui Médenine group of camels. Accordingly, Guiloufi, Merzougui and Ourdhaoui Tataouine camel groups had the higher thoracic girth and the higher height at the withers in comparison with Ourdhaoui Médenine. Ourdhaoui Médenine and Ourdhaoui Tataouine camel groups had the higher tail length compared to Gueoudi, Guiloufi and Merzougui camel groups. The clustering of the five groups allowed describing three main classes of Maghrebi camel including the big, medium and small size Maghrebi camels. According to the coat color, six vernacular names were identified included Chagra, Chaâla, Safra, Hajla, Hamra and Zarga. The quality of the hair was on majority rough and thick, that way the female camel was named Nagga Chalfi. One small proportion was attributed for the females that had sleek and heavy hair which were named Nagga Khawar.

Key words: Maghrebi camels, Tribal affiliation, Body measurements, Hair quality, Southern Tunisia

Introduction

Across the world, there were some reports done on the phenotypic diversity of camel populations, like those of Hülsebusch and Kaufmann (2002) in Northern Kenya, Ishag et al. (2011) in Sudan, Faye et al. (2011) and Abdallah and Faye (2012) in Saudi Arabia. Body measurements revealed clear morphological differences among the local camel population (Hülsebusch and Kaufmann, 2002). A recent phenotypic classification study of Saudi Arabian camel using body measurements revealed 4 types of female camel conformation, 2 breeds and six groups of males (Abdallah and Faye, 2012). The color is a common character used for camel's classification. Based on their coat color, three main breeds of Saudi camels were distinguished, namely Magaheem, Magateer and Al-Homr or Al-Sofr (Wardeh, 1991). Accordingly, Abdallah and Faye (2012) reported that Saudi Arabia camels can include twelve camel phenotypes breeds.

In Tunisia, there are some 1,00,000 camels reared in the arid and desert zones where more than 83% of these camels are found in Tataouine, Kébili and Médenine Governorates (Hammadi, 2003; Sghaier and Moslah, 2004). Despite their significance number, there is relatively limited information available on their genetic diversity (Ben Salah et al., 2009; Ould Ahmed et al., 2010)

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and no clear classification exists, but generally they can be classified according to habitat and function. Two groups of dromedary camels are documented in Tunisia named camel of Tell region and camel of River region. Otherwise, four groups of camel breeders where reported: camel of the South, camel of the Cap-Bon, camel of Sahel and camel of Kairouan (FAO and UNDP, 2000). However, classification on other parameters as the general conformation and body measurements was not clearly described for Maghrebi camels elevated in the arid region of Tunisia. The objective of the current study was to investigate differences of body measurements in relation with the different groups in female Maghrebi camels in Southern Tunisia.

Materials and Methods

Study area

The study, which lasted 9 months from June to February, was achieved in the Southern Tunisia (Figure 1). Southern Tunisia is a transitional region towards the Sahara and includes low plains spreading south of the high steppes, from the Algerian border to the Gulf of Gabès. It can be divided in Matmata Mountain, Jeffara, Dahars and Nefzaoua regions. The Matmata Mountain is small ranges reaching up to 600 m in elevation, enclosed in vast flat stretches of land. The Jeffara region in south-eastern Tunisia is the most southern pastoral region. It lies on the northern fringe of the Sahara desert and has all the features of an arid region and received an annual rainfall between 150 and 200 mm. The Dahars region is characterized by the scarcity of water. It is occasionally inhabited by pastoralists and represents a very important grass reservoir to which many camel flocks from bordering regions move during wet period. The Nefzaoua region is situated in the southwest of Tunisia under arid climatic conditions where the annual mean precipitation is 100 mm and the temperature exceeds 40°C in summer (Belloumi et al., 2006).

In these regions, the extensive livestock breeding stills traditional and is the most common breeding system among small and medium holders that use the local breeds of sheep, goat and camels and make use, above all, of the natural vegetation without any form of management. During autumn season, camels grazed salt pasture (*Sebkha*) and watered from well once each two days depended mainly on the water contained in browsing plants. In spring, all herds of camels was returned towards the pasture of Dahars and did not watering during the period of transhumance. Whereas in the dry season, the camels were watered from well water once in a week and the food was added for animals.

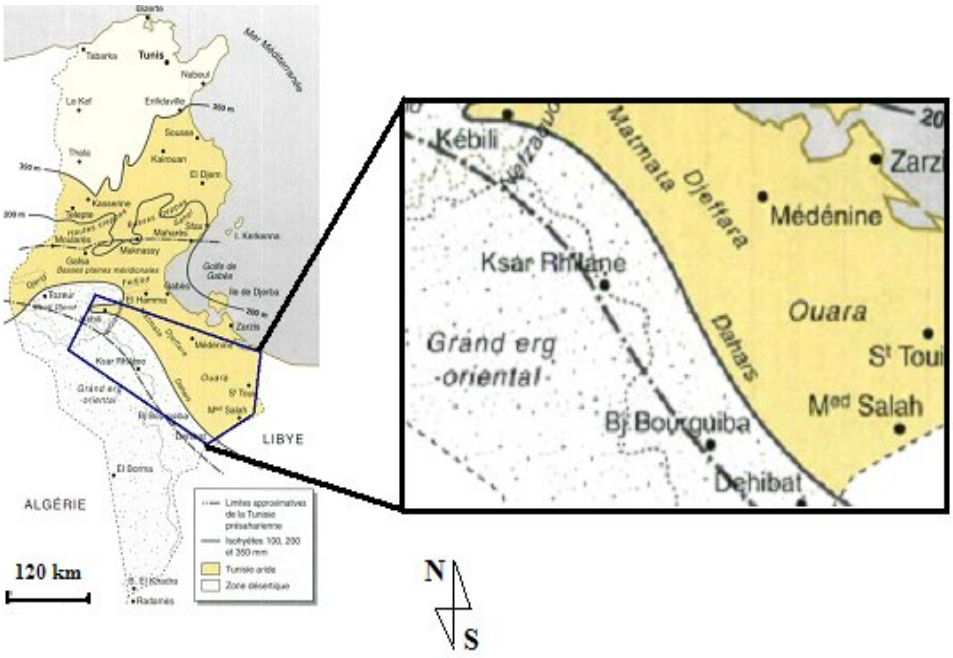

Figure 1. Localization of study area.

Camel herds and body measurements

At the base of preliminary information collected from the national organizations for the Livestock Development (*Office d'Elevage et des Pâturages, Commissariats Régionaux au Développement Agricole)* and camel-breeder, five groups of Maghrebi camel have been identified in the southern Tunisian region. These groups included the Ourdhaoui Médenine (OM) reared by the community of Tawazins, the Ourdhaoui Tataouine (OT) reared by the community of Oudarna, the Guiloufi (GuiK), the Gueoudi (GueK) and the Merzougui (MK) reared in Kébili by the communities of Beni Guilouf, Ouled Gharib and Marazigues, respectively.

The access to camel herds at their grazing area was accomplished after the collaboration with national organizations for the Livestock Development of Médenine, Tataouine and Kébili. The identification of the general data of breeding (breed, colour, breeding practice…) was collected after conversation with camel-breeders and shepherds. A total of 304 not pregnant female camels aged from 6 to 14 years were studied by recording nine body measurements in a standing position using a ribbon meter. Body length (BL) was measured from the shoulder *Pars Caudalis Tuberculi Majoris* to the hip *Tuber Ischiadicum*. The neck length (NL) was also taken as the distance separated the attachment point of the neck at the thorax and the head. The thoracic girth (TG) was measured directly behind the sternal pad. The abdomen circumference (AC) was taken at level of the hump. The height at the hump (HH) was measured from the ground to the top end of the hump, the height at the withers (HW) was measured from the ground to the top end of the *Vertebra thoracica 3*, width at the shoulders (WS) was taken between the two shoulders, the anterior limb length (ALL) was measured from the ground to the level of the shoulder and finally the tail length (TL) was determined from the point tail starting to the end of the rear vertebrae column.

Statistical analyses

To identify sources of variation, data from body measurements were analyzed using SAS package. The significant difference between means of body measurements was tested using Student-Newman-Keuls test. The correlations between the different measurements were assessed by calculating the correlation coefficient of Pearson. The Distance procedure was performed to obtain a distance matrix that was used as input to a subsequent clustering procedure. Frequencies distribution was calculated using Proc Freq. Results are expressed as mean (± standard deviation). The level of significance was set at $P<0.05$ in all analyses.

Results

Body measurements

Variability of all traits was less than 20% (Table 1). Tail length was the most variable trait with a coefficient of variation superior to 15%. The coefficients of variation of the other measurements varied between 5% and 11% (Table 1).

Correlations between the various traits varied between 0.12 and 0.73 (Table 2). The highest correlation coefficients were found between height at the hump (HH) and height at the withers (HW) (0.73; $P<0.001$), between the thoracic girth (TG) and the abdominal circumference (AC) (0.43; $P<0.001$), between the body length (BL) and the neck length (NL) (0.38; $P<0.001$). The lowest positive correlation (0.12) was found between height at the hump (HH) and body length. However, the tail length (TL) was negatively correlated with the abdominal circumference AC (- 0.31; $P<0.001$) and the height at the withers (HW) (- 0.12; $P<0.05$).

Table 1. Mean, standard deviation (S.D), coefficient of variation (C.V) and variance analysis for 9 body measurements and effect of tribe's affiliation group of female Maghrebi camel in Southern Tunisia.

Body measurements	Mean (cm)	S.D	*C.V (%)*	F	Significance
Body length	140	11	7.64	9.4	***
Neck length	101	7	7.40	2.28	ns
Thoracic girth	198	12	6.06	7.16	***
Abdominal circumference	212	23	10.7	62.6	***
Height at the hump	190	10	5.30	8.37	***
Height at the withers	175	10	5.66	14.17	***
Width at shoulders	50	5	9.24	7.58	***
Anterior limb length	123	10	8.49	2.59	*
Tail length	46	7	15.60	55.86	***

***: P<0.001, *: P<0.05, ns: P>0.05.

Table 2. Correlations between body measurements in female Maghrebi camel in Southern Tunisia.

Maghrebi female camels (n = 304)								
	NL	TG	AC	HH	HW	WS	ALL	TL
Body length (BL)	0.38^{***}	0.16^{**}	0.28^{***}	0.12^{*}	-0.01^{ns}	0.11^{ns}	0.21^{***}	-0.10^{ns}
Neck length (NL)		0.20^{***}	0.06^{ns}	0.20^{***}	0.09^{ns}	0.21^{***}	0.28^{***}	0.25^{***}
Thoracic girth (TG)			0.43^{***}	0.31^{***}	0.15^{*}	0.17^{**}	0.07^{ns}	-0.03^{ns}
Abdominal Circumference (AC)				0.41^{***}	0.30^{***}	0.13^{*}	0.06^{ns}	-0.31^{***}
Height at the hump (HH)					0.73^{***}	0.34^{***}	0.14^{*}	0.01^{ns}
Height at the withers (HW)						0.25^{***}	0.14^{*}	-0.12^{*}
Width at shoulders (WS)							0.30^{***}	0.28^{***}
Anterior limb length (ALL)								0.05^{ns}
Tail length (TL)								1^{***}

***: P<0.001,**: P<0.01, *: P<0.05, ns: P>0.05.

There were high significant differences (P<0.001) between the five studied groups of Maghrebi camel, in the following measurements: the body length, thoracic girth, hump circumference, height at the hump, height at the withers, width at the shoulders and tail length. Body measurements according to the camel groups are summarized in Table 3. The body length of Gueoudi (Photo 1) and Guiloufi camel groups (Photo 2) was higher (P<0.05) than in Ourdhaoui Tataouine (Photo 4) and Ourdhaoui Médenine groups (Photo 5). The Merzougui group (Photo 3) occupied an intermediate position between the two groups. The thoracic girth was more important (P<0.05) in Gueoudi, Guiloufi, Ourdhaoui Tataouine and Merzougui camel groups than Ourdhaoui Médenine. The Gueoudi, Merzougui and Guiloufi groups exceeded (P<0.05) the Ourdhaoui group in the abdominal circumference. The Ourdhaoui Médenine group had the small size. The height at hump was more important (P<0.05) in Gueoudi, Guiloufi, Ourdhaoui Tataouine and Merzougui groups than Ourdhaoui Médenine.

Photo 1. Gueoudi Maghrebi camel.
El-Faouar, Rejim Maatoug regions at Kébili.

Photo 2. Guiloufi Maghrebi camel.
Bazma, El Golâa, Bir Aguèreb regions at Kébili.

Photo 3. *Merzougui* Maghrebi camel. Douz, Nowyel regions at Kébili.

Photo 4. *Ourdaoui Tataouine* Maghrebi camel. Daghssen, Remada,Theheeba regions at Tataouine.

Photo 5. *Ourdaoui Médenine* Maghrebi camel. Echoucha, El-Ouâra regions at Médenine.

Table 3. Body measurements (BM) of Gueoudi (GueK), Guiloufi (GuiK), Merzougui (MK), Ourdhaoui Tataouine (OT) and Ourdhaoui Médenine (OM) camel groups. [a,b,c] Means within line with different superscript differ (P<0.05).

Camel groups					
BM (cm)	GueK (n=34)	GuiK (n=38)	MK (n=28)	OT (n=97)	OM (n=107)
BL	147 ± 6 [a]	143 ± 10 [a]	142 ± 6 [ab]	138 ± 9 [bc]	136 ± 13 [c]
NL	100 ± 5	103 ± 8	100 ± 6	99 ± 8	101 ± 8
TG	200 ± 11 [a]	200 ± 9 [a]	199 ± 9 [a]	201± 13 [a]	194 ± 12 [b]
AC	233 ± 8 [a]	226 ± 11 [a]	228 ± 13 [a]	217 ± 19 [b]	193 ± 19 [c]
HH	194 ± 4 [a]	193 ± 7 [a]	190 ± 9 [a]	192 ± 9 [a]	186 ± 12 [b]
HW	182 ± 6 [a]	177 ± 6 [b]	176 ± 8 [b]	177 ± 7 [b]	170 ± 12 [c]
ALL	126 ± 4	126 ± 7	122 ± 5	12 1 ± 15	122 ± 9
WS	51 ± 4 [a]	48 ± 5 [b]	47 ± 3 [b]	51 ± 5 [a]	49 ± 4 [ab]
TL	38 ± 3 [b]	41 ± 6 [b]	39 ± 5 [b]	49 ± 5 [a]	50 ± 6 [a]

BL: Body length; NL: neck length; TG: thoracic girth; AC: abdominal circumference; HH: height at the hump; HW: height at the withers; WS: width at shoulders; ALL: anterior limb length; TL: tail length.

Table 4. Distance matrix calculated between Maghrebi camel groups according to their body measurements.

Camel groups	Geoudi	Guiloufi	Merzougui	Ourdhaoui Tataouine
Geoudi	0.000	---	---	---
Guiloufi	3.085	0.000	---	---
Merzougui	3.594	2.874	0.000	---
Ourdhaoui Tataouine	3.967	4.236	3.318	0.000
Ourdhaoui Médenine	6.378	5.150	4.364	4.282

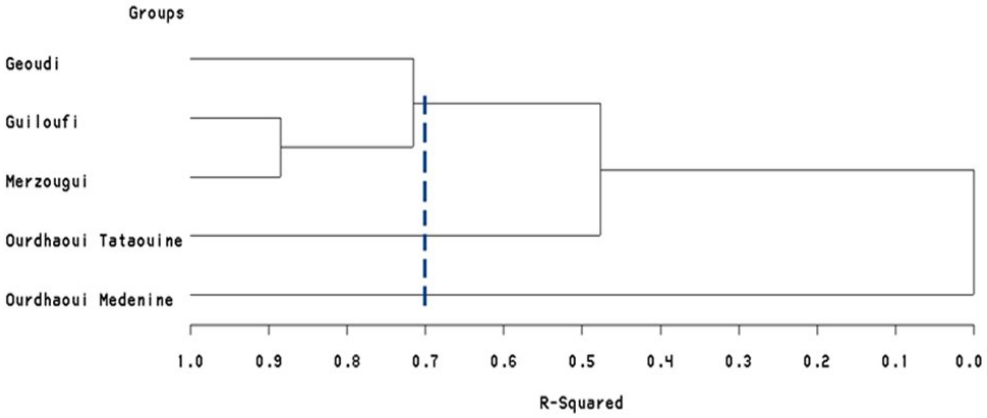

Figure 2. Hierarchical clustering versus R-Square values of Maghrebi camel groups according to 9 body measurements.

Results show that Gueoudi group exceeded (P<0.05) the all others groups in the height at the withers. The Ourdhaoui Tataouine, Guiloufi and Merzougui groups had a medium values but exceeding (P<0.05) the Ourdhaoui Médenine. The width at the shoulders was larger (P<0.05) in Ourdhaoui Tataouine and Gueoudi than Guiloufi and Merzougui camel groups. The Ourdhaoui Médenine group had a medium measurement. The Ourdhaoui group exceeded (P<0.05) Guiloufi, Merzougui and Gueoudi camel groups in the tail length.

The hierarchical classification of body measurements performed on five groups of the female Maghrebi camels was optimal with a partition within 3 classes which explained 70 % of the variance (Table 4 and Figure 2).

A first class (A) included Geoudi, Guiloufi and Merzougui camel groups. They were characterized by the higher measurements of BL, NL, TG, AC, HH, HW and ALL, but with the low measurements of TL and WS. A second class (B) gathered the group of the Ourdhaoui Tataouine which had the medium measurements of BL, NL, AC, ALL and TL. This class had higher measurements of the TG, HH, ALL and WS. The class of Ourdhaoui Médenine (C) was separated from the two others classes and had the low measurements of BL, TG, AC, HH, HW and WS, but with higher length of the neck and the tail (Table 5).

Table 5. Mean body measurements (BM) of the 3 clusters of the groups of female Maghrebi camels.

Classes			
BM (cm)	A (n=100)	B (n=97)	C (n=107)
BL	144 ± 8 ^{a}	138 ± 9 ^{b}	136 ± 13 ^{b}
NL	101 ± 7 ^{a}	99 ± 8 ^{b}	101 ± 8 ^{a}
TG	200 ± 10 ^{a}	201± 13 ^{a}	194 ± 12 ^{b}
AC	229 ± 11 ^{a}	217 ± 19 ^{b}	193 ± 19 ^{c}
HH	192 ± 7 ^{a}	192 ± 9 ^{a}	186 ± 12 ^{b}
HW	179 ± 7 ^{a}	176 ± 7 ^{b}	170 ± 12 ^{c}
ALL	125 ± 6 ^{a}	121 ± 15 ^{b}	122 ± 9 ^{b}
WS	49 ± 4 ^{b}	51 ± 5 ^{a}	49 ± 4 ^{b}
TL	40 ± 5 ^{c}	48 ± 5 ^{b}	50 ± 6 ^{a}

a,b,c Means within line with different superscript differ (P<0.05).

Photo 6. *Nagga Chagra*

Photo 7. *Nagga Chaâla*

Photo 8. *Nagga Safra*

Photo 9. *Nagga Hajla*

Photo 10. *Nagga Hamra*

Photo 11. *Nagga Zarga*

Photo 12. *Nagga Chalfi (or Harcha)*

Photo 13. *Nagga Kawar*

Qualitative traits

Our study revealed that the colour range of female Maghrebi camels is very heterogeneous. According to camel-breeders and shepherds, six vernaculars colours were described and varied between the white at the grey and namely, *Chagra* (white: 6.5%, Photo 6), *Chaâla* (slightly white: 5.5%, Photo 7), *Safra* (yellow: 28.9%, Photo 8), *Hajla* (fawn: 8.6%, Photo 9), *Hamra* (brown: 29.6%, Photo 10) and *Zarga* (gray: 20.9%, Photo 11).

The main type of hair in camels was rough (97.7%), whereas the sleek hair was found only in some females (2.3%). Often, the female camel with a rough hair is named *Nagga Chalfi or Harcha* (Photo 12), whereas that with a sleek hair is named *Nagga Kawar* (Photo 13). In fact, the *Nagga Kawar* is known by a good milk potential but the camel owners generally preferred *Chalfi* because she is very adapted to the harsh conditions in arid and desert regions.

Discussion

The present study provides body measurements in female Maghrebi camels. The body length measured in Maghrebi camel was less than those described for Gabbra camels in Somali, for Meghem, Gamra and Awadi Arabian camels in Saudi Arabia (Al Hazmi et al., 1994; Hülsebusch and Kaufman, 2002). Furthermore, the Mewari and the Jaisalmeri camel breeds had a more long body (Mehta et al., 2007) in comparison with Maghrebi camels. The Maghrebi camels showed similar measurements of thoracic girth in comparison with the Arabian camels (Al Hazmi et al., 1994), Mewari and Jaisalmeri camels (Mehta et al., 2007). The height at the withers was higher in the Maghrebi camel than in the Rendille camel, but smaller than those measured in Mewari, Jaisalmeri and Targui camels (Mehta et al., 2007; Oulad Belkhir et al., 2013). These differences between groups of dromedary in term of body measurements are genetically linked and revealing geographical distribution (Mahrous et al., 2011; Almathen et al., 2012). The height at the hump was low in Maghrebi camels compared to those described for Sahroaui and Targui in Algeria (Oulad Belkhir et al., 2013). The hump size varies according to the body condition score of the animal (Kamili et al., 2006). Indeed, the hump is the main fat storage form in camel representing on average 85% of the adipose tissue (Faye et al., 2001).

In our study, the comparisons reveal clear differences between five groups of Maghrebi camels. The groups of Gueoudi, Guiloufi and Merzougui showed the largest body measurements compared to the Ourdhaoui Tataouine and Ourdhaoui Médenine. The clustering of the different groups allowed describing three main classes of Maghrebi camel. These classes could be described: i) the big size camels from the areas of Kébili and which included the Geoudi, Guiloufi and Merzougui groups; ii) the medium size camels (Ourdhaoui Tataouine) from the regions of Tataouine; iii) the small size camels (Ourdhaoui Médenine) from the regions of Médenine. This grouping of Maghrebi camels revealed differences which are mainly linked to geographical distribution as it has been observed in previous study (Ould Ahmed et al., 2010).

The study of hair revealed that the colour of Maghrebi camel groups range was very heterogeneous and six vernaculars colours have been revealed for the five studied groups. Those results differ from that have been revealed for Rendille camels of Kenya known by two roan colours, namely *wersi* and *borakhan* and two solid colours *surwa* and *gatab hass*. The colour *gomboch* could not be found in the sample but might be identical to *gatab hass*, as it is described as a light yellow. The new colours account for less than 0.8% of the total sample while the colours *bori* (brown), *dakhan* (white) and *eidimo* (dark fawn) together add up to 67.4% of the all colours within the sample (Adams and Kaufmann, 2003). Otherwise, each breed of Arabian camel has its own colour: the Meghem breed has black hair, the Sawahli breed is light and the Awadi breed darken in the hump; while the Gamra breed is whiter than the others (Al Hazmi et al., 1994). Accordingly, Abdalla and Faye (2012) described twelve different colors in camel breeds from Saudi Arabia.

Conclusion

Phenotypical description of Maghrebi camels according to their tribal affiliation in southern Tunisia could be a first contribution for classification of the different groups of camel, and for valorising their genetic biodiversity. A clear description of camel population is an important step, especially with current changes in their farming systems (settlement, intensification, indoor feeding systems, implementation around the towns, etc.,). For this reason, the present study limited to the body traits of Maghrebi camels in according to their tribal affiliation would be an opportunity to underline the richness of this emblematic animal whatever their social rank or the place where they

are living. The results reported the existence of subgroups among the local population. However, for further refining this study, it is necessary to consider the performance characteristics (meat production, milk productivity and numerical productivity) and variation analyze in DNA fingerprinting, with the perspective to lead to a kind of standards Maghrebi camels.

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AGRICULTURAL ECONOMICS

Market sustainability analysis of jenny milk cosmetics

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Abstract

A quantitative market analysis was carried out to deepen the knowledge on some qualitative and quantitative aspects of jenny milk and cosmetics and, successively, a survey was carried out to study the influence of packaging on consumer liking. The first analysis was conducted using the quantitative method by interviews with a one to one questionnaire, consisting of 18 questions. In the second survey were interviewed 300 people with a questionnaire divided into two perception areas: of packaging paper, and of packaging communication. Results showed that 67.8% of consumers had familiarity with jenny milk, but only 19.5% knew cosmetic properties of this product. The panel expressed a low expenditure propensity toward the proposed cosmetics. For these products, the study on packaging identified a type of matte paper as the favorite by consumer. Moreover, panel judged the icon of donkey and naming Asinella as the most persuasive.

Key words: Cosmetics survey, Jenny milk, Matte packaging, Exalt cosmetics

Introduction

During the nineteenth century, chemicals were used to replace more expensive natural ingredients making the cosmetics more widely used. Nevertheless, the growing interest in health of consumers is nowadays reversing this trend involving an increased interest related to natural ingredients. Even more, the 21st agenda item of World Summit carried out in Rio in 1992 emphasized the importance of the sustainability of industrial production. In recent years, the cosmetic industry is mainly focused towards products made with natural ingredients, and it is oriented to a sustainable consumption. Many cosmetics industries developed publicity campaigns with images of an eco-friendly company to promote their cosmetics. In addition, the marketing becomes attentive to sustainability increasingly in the creation and production of the packaging product by the study of consumer behavior.

To increase sustainable patterns of consumption, it is important to understand more on purchasing decision of consumers. Despite the consolidated positive trend of natural cosmetics, there are few reports on the qualitative and quantitative market analyses for jenny milk cosmetics. These studies regard principally their moisturizing and rebalancing properties on the membranes of skin cells. It is known that these properties are principally due to the high lysozyme and to the antioxidant action of fatty acids contained in jenny milk (Herrouin et al., 2000; Polidori et al., 2009; Restani et al., 2009; Tesse et al., 2009; Simos et al., 2011; Al-Saiady et al., 2012; Cosentino et al., 2012a,b). Few reports demonstrated that jenny milk prevents skin-aging process hydrating and restructuring the dermal intercellular substance (Guo et al., 2007; Orsingher, 2011; Paolino et al., 2011).

The willingness of consumers to purchase such products is usually influenced by cosmetic composition and ingredients (Kuznesof et al., 1997; Power, 2010; Khraim, 2011). The quality cannot be experienced before the consumer purchase, but it is possible to communicate it thanks to extrinsic indicators (brand, origin, nutritional information, composition, label, price) (Grunert et al., 2000). In this contest, the functions of packaging are to enhance and to transmit the information of product, facilitating use and transport (Qing et al., 2012). Currently, packaging heavily impacts the perceived quality of products, and is often considered to be as important as the product (Raheem, 2013). However,

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few studies have been conducted on the sensory properties of packages (Lefebvre et al., 2010). The measurement of packaging attributes is very difficult because a package generally induces a wide variety of stimuli: visual, tactile, and even olfactory. For example, visual and tactile stimuli, affect the consumer choices at time of purchase. The communicative function of packaging is supported by iconic and textual elements represented by brand and naming (Topoyan et al., 2008). Moreover, the marketing of these products could help to preserve, at the same time, local donkey genotypes and the marginal areas in which they are reared. This study was conducted to provide preliminary data from Basilicata, a region of South Italy, related to consumer knowledge on jenny milk cosmetics. The objectives of this study were to evaluate the consumer knowledge on some qualitative and quantitative aspects of jenny milk cosmetics, and to identify some packaging factors that influence consumer liking.

Materials and Methods

Experiment 1: Market survey

This descriptive study was conducted using the quantitative method by interviews with a *one to one* questionnaire that has been randomly administered to 450 people residing in the test area; of these, only 392 people have correctly completed the questionnaire. Therefore, the sample was composed by 181 men and 211 women, ageing, in mean, 40.04±11.30 s.d. years. Since people in this age range are the most strongly concerned to counteract skin-aging process and the appearance of wrinkles, in this preliminary test, we did not consider other age groups. A questionnaire consisting of 18 questions was utilized (Table 1).

In order to test the questionnaire, a previous validation was effected utilizing 30 persons, recruited in a supermarket of Potenza (55% women, and 45% men, with a age range of 18-60 years).

Experiment 2: Packaging survey

The consumers involved in this study were selected from 3,400 people that, within a year, purchased at least 2 packages of all the following products: face cream, body lotion, hand cream, moisturizer, and lip cream after sun or sunscreen. In particular, the average number of product/year purchased by the selected consumers was: 3.21 face cream, 2.9 bodies cream, 3.29 hand creams, 2.76 lips moisturizer, 2.83 sunscreen. The selected consumers, 300 people, (48% men and 52% women) aged, in mean, 33 years ±11.14 years.

The naming has been developed starting from the concepts of "delicacy" of "naturalness". Therefore was preferred the direct representation of the product source: the donkey, giving it a specific soft brown tone coat. A jury of 50 habitual cosmetic consumers (78% women, and 22% men, aged range 18-54 years) interviewed in 3 perfumeries of Potenza preferred the naming “Asinella” to the naming “Asinus” and “Donkey”; even the farmers identified it as the best choice (Figure 1).

Figure 1. Naming Asinella.

The questionnaire of packaging survey was divided into two analysis areas: 1) liking of the type of packing paper (matte; glossy; glossy embossed with texture), and 2) communication aspect of packaging (A, “Asinella”; B, “Asinella” + “Natural product”; C, “Asinella” + ”Natural product” + “Made in Basilicata”) (Figure 2). The questionnaire was tested and validated with a pilot test employing 15 people, recruited from University staff (58% women, and 42% men, age range 30-61 years). To evaluate the preferences of consumers we used a scale of values from 1 to 5. Comparisons between types of paper and between types of communication aspect were tested by Tukey’s test (SAS, 1999).

Results

Experiment 1: Market survey

This study was conducted for evaluating the degree of knowledge of 392 consumers towards the jenny milk cosmetics. Of these 265 (67.8%) knew jenny milk, and only 52 (19.5%) were already familiar with jenny milk cosmetics. Only 36 (9.2%) of surveyed customers had already bought this product; nevertheless, 247 of them (63%) expressed willingness to buy jenny milk cosmetics in the future. In particular, the most common buying motivation was “curiosity toward product” (in 214 consumers, 54.6%) followed “personal use” (in 151 consumers, 38.6%) and “to utilize as gift” (in 27 consumers, 6.8%).

Table 1. Questionnaire of market survey.

1. In which of the commercial sites listed below do you shop? (one answer) ☐small businesses ☐ supermarket ☐shopping center ☐directly from the producer
2. Are you an habitual cosmetic consumer? ☐ Yes ☐ No
3. Would you like to buy an innovative cosmetics? ☐ Yes ☐ No
4. The signature "only with natural ingredients" on a packaging for a body care cosmetic at what expression listed below make you think of? (one answer)
5. Could positively influence your choice the signature "only with natural ingredients" on a packaging for a body care
6. Do you usually buy only famous brands cosmetic? ☐ Yes ☐ No
7. Usually, where do you buy cosmetics? (one answer) ☐ pharmacy ☐ herbalist's shop ☐ supermarket ☐ perfumery ☐ beauty farm ☐ hairdresser
8. Do you know donkey's milk? ☐ Yes: ☐ in alimentary use ☐ in cosmetic use ☐ in alimentary and in cosmetic use ☐ No: If NO then go to question 14
9. Have you ever bought any food made with donkey's milk? ☐ Yes ☐ No
10. Have you ever bought any jenny milk cosmetics? ☐ Yes, because: (one answer) ☐ I know its properties ☐ for curiosity toward product ☐ I consider it like a natural product ☐ I consider it like an innovative product ☐ No, because: (one answer) ☐ I'm not really interesting in this product ☐ It's not readily available ☐ It's too expensive ☐ I'm wary about its effectiveness
10. How satisfied are you in purchase of jenny milk cosmetics? ☐ really not ☐ not much ☐
12. Recently, which kind of jenny milk cosmetics did you buy? (one answer) ☐ face cream ☐ bubble bath ☐ body
13. Of this product do you remember brand, quantity, and price? Brands_____ Price_____ Quantity
14. Would you buy again jenny milk cosmetics? ☐ YES: ☐ NO: If NO then go to question 14 ☐ for my personal care; ☐ as gift;
15. Indicate two products that you would routinely buy ☐body cream ☐ face cream ☐ hand cream ☐shower cream
16. How much would you spend for jenny milk cosmetics? ☐ body cream, 200 ml ☐ 15 – 25 euro ☐ 25 - 35 euro ☐ over 35 euro ☐ face cream, 50 ml ☐ 20 - 30 euro ☐ 30 - 40 euro ☐ over 40 euro ☐ hand cream, 100 ml ☐ 8 – 15 euro ☐ 15 - 22 euro ☐ over 22 euro ☐ bubble bath, 200 ml ☐ 8 – 13 euro ☐ 13 - 18 euro ☐ over 18 euro
17. Would you be willing to pay an higher cost for a cosmetic produced with milk from jennies reared in Basilicata? ☐ Yes ☐ No
18. Would you be willing to try jenny milk cosmetics? ☐ Yes ☐ No

Figure 2. Packaging text labels.

The buying preferences for cosmetics were, in decreasing in order: body cream (32%), bubble bath (30%), face cream (23%), and hand cream (15%). Even if the surveyed group showed a willingness to buy cosmetics in low price classes (hand cream, 8-15 euro, 81%; body cream, 15-25 euro, 76%; bubble bath, 8-13 euro, face cream, 20-30 euro, 69%) about 68% of them would have purchased jenny milk cosmetics at a higher price if produced in sustainable rearing system in the respect of animal welfare (Figure 3).

The consumers expressed their preference for the following distribution channel: perfume shop (48%), pharmacy (40%), and supermarket (12%).

Experiment 2: Packaging survey

Perception of the paper

The "matte paper" resulted the most appreciated (4.03, $P<0.05$). In fact, according to the surveyed consumers, "matte paper" evoked the perception of natural more than to the "glossy paper" (2.17), used by Asilac, Milk drops, and Dahl (italian competitors), and more than to the "glossy paper embossed with texture" (2.36). Consequently, the willingness to buy cosmetics packaged with "matt paper" was significantly the highest (4.1, $P<0.05$; 2.56 in "glossy paper" and 2.94 in "glossy paper with embossed texture") (Figure 4).

Perception of packaging

In this section, in question "Do packaging information induce to purchase?" the consumers judged the message of type B more 'persuasive' (4.09, $P<0.05$) than the other two communication forms of packaging (3.31 and 3.9, in packaging A and in packaging C, respectively). In the question "Do packaging information include elements of reliability?" the surveyed consumers judged the type B the most persuasive for the reliability of the product (3.89, $P<0.05$; 2.8 in packaging A and 3.84 in packaging C) (Figure 5).

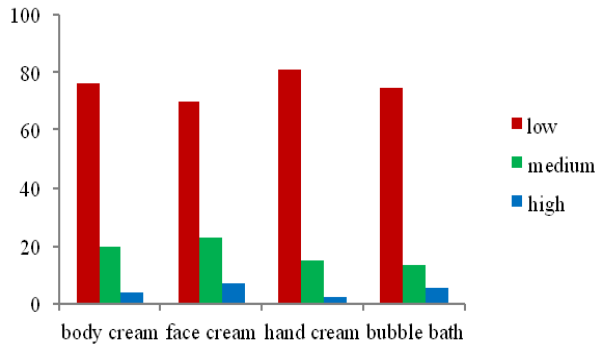

Figure 3. Willingness to pay: Frequency in three price classes (%).

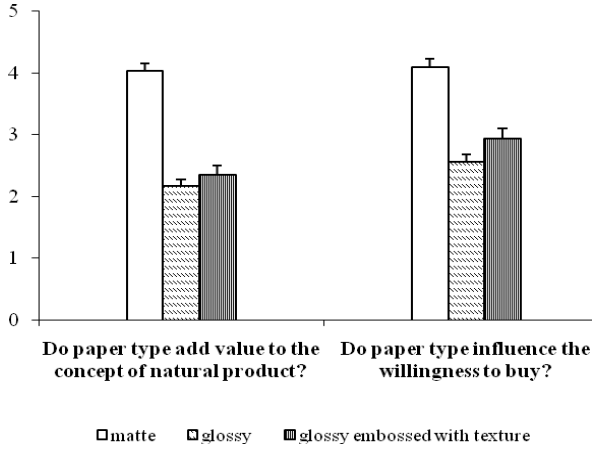

Figure 4. Perception of paper type.

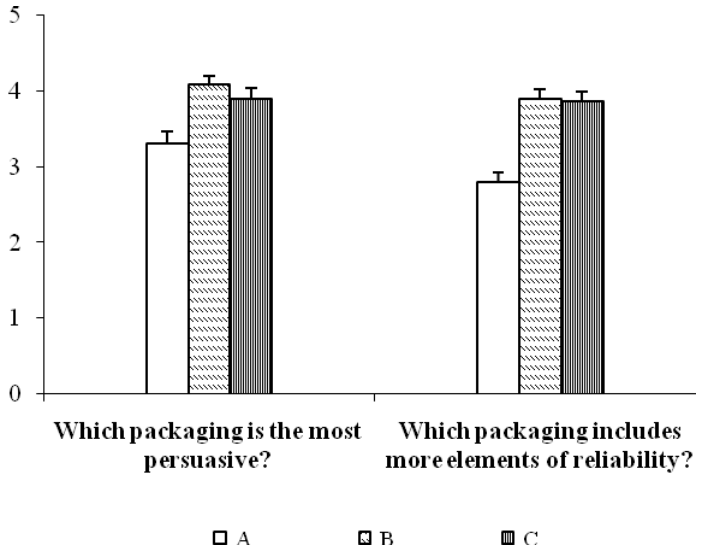

Figure 5. Perception of packaging.

The surveyed consumers considered the most persuasive combination in purchase the naming “Asinella”, with the text label "Natural product". The text label “Made in Basilicata” did not significantly affect the acceptability of the sample survey. Probably, "Made in Basilicata" is not yet perceived as representative of an uncontaminated and natural region, directly linked to the health and naturalness of a product. From the consumer perspective, naming is an important quality cues and makes it easier to infer quality. In addition to this parameter, traceability systems, branding, and labeling can help consumer’s choice (Grunert, 2002).

Conclusion

Our results showed that over 66% of the surveyed consumers are willing to purchase ‘new generation’ cosmetics, probably because the term refers to innovation and modernity in their imagination. These cosmetics could absorb a significant portion of the market for natural cosmetics based on milk. The results of descriptive survey put in evidence that customers knew jenny milk and expressed willingness to buy jenny milk cosmetics in the future. Survey consumers preferred the type of packaging most closely to the concept of "natural". Probably, the expansion of jenny rearing for milk production can increase the availability of milk for making innovative cosmetics. These products can take a placement in the market for natural cosmetics made with low-impact processes in a rural region like Basilicata, in which is widespread the extensive rearing system. Further tests about paper packaging considering different paper colors and text label will be taken into account in order to know the consumer acceptability.

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