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Full length Research Paper

Biochemical changes during storage of chocolate

Poonam Yadav, J. P. Pandey and S. K. Garg*

Department of PHPFE, College of Technology, GBPUAT, Pantnagar.

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In India, consumption of chocolate rank first among common confectionery food and number of industries engaged in processing of chocolate. The temperature ranges varied from 25˚C to 40˚C with an increment of 5˚C and the relative humidity levels were 30, 55 and 75 per cent. The samples (heat sealed packed, butter paper packed and unpacked chocolate) were stored in desiccators having salt solution for maintain relative humidity (30, 55 and 75 per cent) and kept in incubators at experimental temperature (25, 30, 35 and 40˚C). The chocolate quality was detected in terms of moisture content, FFA, and PV at the time of procurement respectively 0.21 per cent db, 0.35 per cent Oleic acids, 0.55 MeqO$_2$/Kg,. After 130 days of storage the maximum value of moisture content, FFA, and PV were respectively 1.74 per cent (db), 1.39 (per cent Oleic acid), 2.27 (MeqO$_2$/Kg Fat), for heat sealed laminated packed chocolate.

Keywords: Chocolate, confectionary, heat sealed packed, butter paper packed.

INTRODUCTION

Chocolate is one of the most popular foods and common confectionery material in the world, people enjoyed for its wonderful taste. Chocolate is a product of cocoa, made by mixing cocoa mass, cocoa butter and sugar (sucrose) using special machinery. In India as well as in Asia the chocolate market catching an increasing trend. In India the consumption of chocolate rank first among common confectionery food. Chocolate was introduced to Europe exclusively in Spain in the 16th and 17th century. The consumption of chocolate was an exclusive privilege for the aristocracy and the clergy at the royal courts. The industrialization of chocolate production began in the beginning of the 20th century but even then it remained an adult luxury product, only for special occasions, celebrations or tender moments between friends (Jyoti 2003).

Chocolate and cocoa are products derived from cacao beans, the seeds of the Theobroma cacao tree. This tree is native to South America, from where it naturally spread into Central America (Bearden et al., 2000). A good chocolate is shiny brown, breaks cleanly, and is free of lumps, tiny burst bubbles, and white specks. It melts on the tongue like butter, has a true aroma of chocolate rather than of cocoa powder, and is neither greasy nor sticky. The taste of chocolate is partially determined by the chemistry of the product; typical formulations of ingredients used manufacturing of chocolate. However, the taste experienced by the consumer also depends critically on the micrometer-scale structure of the chocolate ingredients, which consists of crystals and particles ranging from 10 micro m to 120 micro m in diameter, depending on the product. Taste depends on the release of flavor compounds to the mouth and nose, while perceived texture is a function of the way in which the material melts and breaks up in the mouth. The preference of taste of chocolate varies from country to country Kulozik et al 2003. The present investigation has been carried out on chocolate supplied by private company Limited in the form of heat sealed laminate packing .The unpacking and butter paper packaging is done by investigator. Salunkhe et al 1979 reported in their research work that the chocolate looses its taste and flavor and became rancid during storage. The composition of the chocolate can play an important role on its shelf life (Bernard, 1989) Chocolate without milk can be stored for several months (or even years) if it is protected from damp and stored at 20℃ temperature. Chocolate is very sensitive to temperature and humidity (Karbancioglu, 2004).

Further, the researcher points out, that chocolates should be stored in a `dry place' but they have not given a specific recommendation and also the systematic study

*Corresponding Author E-mail: sanjeevkgarg@gmail.com

on storage of chocolate is not available in the literature (Jyoti, 2003). Keeping in view of above facts the present research work is plan with the objectives: To study the effect of temperature, relative humidity on biochemical changes of chocolate during storage.

MATERIALS AND METHODS

Investigations were conducted to determine the kinetics of moisture absorption and biochemical changes of chocolate during storage at different condition. In the present study chocolates were received from a private company, they were kept at room temperature (15±1°C), until proper storage condition provided.

The experiments were planned with three independent variables, temperature, relative humidity and packaging. Four levels of temperature (25, 30, 35 and 40^{0}C), three levels of relative humidity (30, 55 and 75 per cent) and 3 levels of packaging (heat sealed laminate packing, butter paper packing and unpack) were selected as variables. Samples were stored for 130 days in desiccators having saturated salt solution to maintain relative humidity. Desiccators were kept in incubator to attain constant temperature during storage period. The quality of chocolate was determined in terms of moisture content, free fatty acids and peroxide value for the fresh and 130 days stored chocolate

Data analysis was done for responses based on the independent variables using ANOVA for Randomize Block design (four factors). Analysis of variance represents the significance of observation by comparing calculated F-value and table F-value at desired probability level for the given degree of freedom and the error associated with process conditions. A calculated F-value exceeding the table F-value indicates that significant differences exist among the process conditions.

Moisture content

Five g of sample was dried to a constant weight at 100 °C in a moisture dish for one hour (AOAC, 1969). The loss in weight was used to calculate per cent moisture using following expressionMoisture content, percent

$$= \frac{\text{Loss in weight}}{\text{Weight of sample}} \text{x}100 \text{.......} 1$$

Biochemical analysis

Extraction of Free fatty acid from the chocolate

Extraction of fat of using Soxtec rapid solvent extractor. Fine sample was transferred into thimble and extraction was carried out at 100°C allowing 30 min boiling and 60 min rinsing time. To determine free fatty acid of chocolate fat about 5g fat was weighed, dissolved in about 50 ml of hot alcohol (previously neutralized) and add 1.0 ml of phenolphthalein indicator. Brought it to the boiling point and while still hot, titrate with the standard 0.1 N KOH or NaOH solution shaking vigorously during the titration **(**IS 966: 1999**).** The end point of the titration is reached when the addition of a single drop produces a slight but definite pink colour persisting for at least 15 seconds.

Free fatty acids (as Oleic acid), per cent by mass

$$\text{FFA} = \frac{28.2VN}{M} \text{ .... } 2$$

Where,

V = volume in ml of the standard potassium hydroxide or sodium hydroxide solution used for titration

N = normality of the standard potassium/sodium hydroxide solution,

M = mass in g of the sample taken for the test

Peroxide value

For peroxide value (PV), 1 g of oil/fat extracted from 'chocolate' was taken in a conical flask, 25 ml of solvent was added and the air above the liquid was displaced with CO_2. Then 1 ml of potassium iodide solution was added, allowed to stand for 1 min, 35 ml of water was added and the liberated iodine was titrated with 0.1 N sodium thiosulphate solution using of starch as an indicator (Ranganna, 2005) PV meq/1000 gm fat =

$$\frac{\{\text{Sample titre} - \text{Blank titre}\} \times \text{normality of sodium thiosulphate solution} \times 1000}{\text{weight of fat taken}}$$

RESULTS AND DISCUSSION

The basic ingredients involve for chocolate (Meltz) preparation were sugar, edible vegetable fat, milk solids, cocoa solids, permitted emulsifying and stabilising agent and salt in different concentration. The level of quality parameters in terms of moisture content, free fatty acid and peroxide value at the time of procurement were 0.21 per cent db, 0.35 per cent Oleic acid, 0.55 MeqO_2/ Kg Fat, respectively, which is within the safe limit for consumption of chocolate. The quality of chocolate was determined in terms of moisture content, free fatty acids, peroxide value.

Moisture Content of Chocolate during Storage

The moisture content of chocolate (heat-sealed laminate packing, butter paper packing and unpack) was found

Table 1: ANOVA for moisture content of chocolate stored under different experimental condition

SOURCE	DF	SS	MSS	F_{Cal}	F_{Tab}
REPL	2	0.0491	0.0245	0.0174	3.00
TREAT	251	785.1513	3.1281	2.2119	1.19
Relative Humidity (RH)	2	34.5127	17.2564	12.2023	3.00
Temperature (T)	3	28.4249	9.475	6.6999	2.21
Packaging (P)	2	4.6123	2.3062	1.6307	3.00
Storage Period (S)	6	11.8033	1.9672	1.391	2.10
RH * T	6	290.1553	48.3592	34.1956	2.10
RH * P	4	20.5246	5.1311	3.6283	2.38
RH * S	12	5.6568	0.4714	0.3333	1.76
T * P	6	18.8689	3.1448	2.2238	2.10
T. * S	18	4.3357	0.2409	0.1703	1.58
P * S	12	2.1271	0.1773	0.1253	1.76
RH * T * P	12	160.9273	13.4106	9.4829	1.76
RH * T * S	36	44.9215	1.2478	0.8824	1.41
RH * P * S	24	12.5255	0.5219	0.369	1.53
T.X P * S	36	16.5276	0.4591	0.3246	1.41
RH * T *P * S	72	129.2278	1.7948	1.2692	1.30
ERROR	502	709.9248	1.4142		

(DF = degree of freedom; SS = Sum of Square; MSS = Mean Sum of Square; F_{Cal} = F Calculated and F_{Tab} = F Table value)

0.21 per cent (db) at the initial of storage (0 day). The moisture content varied from 0.21 to 1.28; 0.21 to 1.18; 0.21 to1.23 and 0.21 to 1.28 for 30 per cent relative humidity at 25, 30, 35 and 40^{0}C respectively for heat-sealed laminate packing. While the per cent moisture content of chocolate were 1.54, 1.61, 1.63 and 1.61; 1.74, 2.20, 2.11 and 1.74 for 55 per cent and 75 per cent relative humidity 25, 30, 35 and 40^{0}C respectively.

The moisture content of unpack chocolate ranged 0.21 to 3.53, 0.21 to 1.71, 0.21 to 1.69 and 0.21 to 3.12; 0.21 to 3.65, 0.21 to 3.79, 0.21 to 3.82 and 0.21 to 4.76 and 0.21 to 4.10, 0.21 to 6.93, 0.21 to 8.02 and 0.21 to 8.35 per cent for the relative humidity at 30, 55 and 75 per cent at 25, 30, 35 and 40^{0}C respectively. The maximum moisture absorption was found in the unpacked chocolate at 75 per cent relative humidity for all experimental temperature. It reveals that the chocolates with different packaging absorb moisture in increasing trend with storage period for all experimental combinations. The maximum moisture content was found at the end of storage period for all (heat-sealed laminated packaging, butter paper packed and unpacked) chocolate.

Table 1 showed the analysis of variance for chocolate moisture content indicated that the effect of relative humidity, temperature and packaging on moisture content are significantly different at 5 per cent level of significance (F_{Cal} > $F_{Tab)}$ where as storage period have less influence on moisture content at the same level of significance. The 30 per cent relative humidity for temperature 25, 30, 35 and 40^{0}C the heat sealed laminated packaging had minimum absorption of moisture even at the end of 130 days of storage period. Similar observation also observed at higher (75 per cent) relative humidity for these temperatures.

The maximum moisture gain was 3.89,6.72,7.81 and 8.14 was observed at 75 per cent relative humidity for 25, 30, 35 and 40^{0}C in case of unpacked chocolate, while at 30 per cent relative humidity for temperature 25,30,35 and 40^{0}C total moisture gain was found 3.32,1.5,1.02 and 1.07 per cent in unpack chocolate. The butter paper packed chocolate the total moisture gain at the end of 130 days in-between unpack and heat sealed for 30, 55 and 75 per cent relative humidity at all experimental temperature. The chocolate stored at high temperature 35 and 40^{0}C and high relative humidity more than 55 per cent showed a higher absorption of moisture**.** Karbancioglu (2004) has reported similar observations**.**

The interrelationship between storage period and moisture content of chocolate (heat-sealed laminated, butter paper packaging and unpacked) exposed in different condition was correlated. The correlation shows the kinetics of moisture absorption by chocolate. The three non-linear mathematical models (logistical, exponential and bolzmann) were used to correlate the relationship. The Bolzmann model showed higher 'R^2' value and lower value of SEE for all types of chocolates. Hence the Bolzmann model was selected which predict most precisely kinetics of moisture behaviour of chocolate. The Boltzmann model has the following equation;

Table 2: ANOVA for FFA of chocolate stored under different experimental condition

ANOVA FOR RBD (FOUR FACTOR)					
SOURCE	**DF**	**SS**	**MSS**	**F_{Cal}**	**F_{Tab}**
REPL	2	0.0486	0.0243	13.2088	3.00
TREAT	251	1098.513	4.3765	2378.9	1.19
Relative humidity (RH)	2	30.0744	15.0372	8173.569	3.00
Temperature (T)	3	1.8397	0.6132	333.3289	2.21
Packaging (P)	2	212.848	106.424	57847.47	3.00
Storage Period (S)	6	640.9069	106.8178	58061.53	2.10
RH X T	6	10.4574	1.7429	947.3647	2.10
RH X P	4	10.6034	2.6508	1440.881	2.38
RH X S	12	13.6196	1.135	616.9196	1.76
T.X P	6	0.7455	0.1243	67.5382	2.10
T X S	18	1.4006	0.0778	42.2946	1.58
P X S	12	136.9308	11.4109	6202.469	1.76
RH X T X P	12	9.2914	0.7743	420.8683	1.76
RHX T X S	36	11.7112	0.3253	176.8248	1.41
RH X P X S	24	6.5406	0.2725	148.1327	1.53
T.X P X S	36	2.2414	0.0623	33.8429	1.41
RH X T X P X S	72	9.302	0.1292	70.2245	1.30
ERROR	502	0.9235	0.0018		

(DF = degree of freedom; SS = Sum of Square; MSS = Mean Sum of Square; F_{Cal} = F Calculated and F_{Tab} = F Table value)

$$Y = \frac{A1 - A2}{1 + e^{(X - A0)/dx}} + A2 \quad .... 3$$

Where;
X = Storage time, days
Y = Moisture content, per cent db
dx = Small time interval
A_1 and A_2 are constants
A_0 = (A1+A2)/2

The R^2 value ranged 0.957 to 0.999, 0.95 to 1.00 and 0.952 to 0.996 whereas SEE varied 0.037 to 0.00, 0.268 to 0.00 and 0.333 to 0.003 for heat-sealed laminated, butter paper and unpacked chocolates exposed in experimental condition.

Effect of Storage on Free Fatty Acid in Chocolate

The Free Fatty Acid (FFA) is the primary quality attribute for edible grade oil / fat. The Purified Food and Adulteration Act specify a maximum acceptable limit of FFA as 3 per cent (Ranganna, 2005). The unpleasant odour and taste which develops spontaneously in fats, known as rancidity, is of two types: hydrolytic and oxidative. Oxidative rancidity is due to oxidation of double bond of fatty acid with the formation of aldehydes, ketones and acid of lower molecular weight than the fatty acid originally present. The process depends on presence of oxygen; it is hastened by heat light, moisture and certain metal catalysts. Oxidative rancidity is due to chiefly to the oxidation of oleic acid (Ranganna, 2005). The FFA value has been expressed as percent oleic acid. The minimum value (0.35) of per cent oleic acid was found at the time of start of storage of different pack of chocolate. The rise of FFA was recorded maximum at 75 per cent relative humidity for all four experimental temperatures. The minimum (1.23, 1.28, 1.28 and 1.54) increase in FFA values were observed with heat-sealed laminated packaging at 30 per cent relative humidity for 25,30, 35 and 40^{0}C respectively. Where as the FFA of chocolate for 55 and 75 per cent relative humidity were 1.25, 1.29, 1.32 and 1.30; 1.41, 1.37, 1.38 and 1.39 at 25, 30, 35 and 40^{0}C respectively for heat-sealed laminated packing.

The FFA for unpacked chocolate was higher than packed (butter paper and heat-sealed laminated) chocolates. FFA values was found for unpacked chocolate was 2.98, 3.14, 3.38 and 3.99; 3.78, 3.82, 3.96 and 4.53; 3.97, 4.26, 4.62 and 4.98 for the relative humidity at 30, 55 and 75 per cent at 25, 30, 35 and 40^{0}C respectively. Butter paper packed chocolates showed the rise of FFA value in between heat sealed laminated packed and unpacked chocolate. Table 2 showed the analysis of variance for FFA of chocolate stored under different experimental condition in Randomised block design. The analysis of variance showed that all experimental combinations are significantly different over treatment ($P<0.05$). ANOVA indicated that at 5 per cent level of significance the temperature, relative humidity and their interaction can influence the rise of FFA similar observation have recoded by Karbancioglu (2004) and Ali et al (2001).

Table 3: Peroxide Value of chocolate at different relative humidity and temperature during storage

S.No.	RH %	Packaging	PV ($MeqO_2$/ Kg Fat) Storage Period (days)						
			0	30	50	70	90	110	130
			Storage Temperature 25^{0}C						
1		Hl	0.55	0.77	0.97	1.25	1.52	1.75	1.92
2	30	Bp	0.55	0.85	1.11	1.59	1.78	2.95	3.48
3		Up	0.55	0.89	1.42	2.91	3.41	4.12	4.66
4		Hl	0.55	0.83	1.00	1.34	1.45	1.81	1.95
5	55	Bp	0.55	0.86	1.27	2.18	3.39	4.14	4.94
6		Up	0.55	0.89	1.36	3.09	3.69	4.64	5.91
7		Hl	0.55	0.81	1.05	1.33	1.53	2.13	2.20
8	75	Bp	0.55	0.88	1.81	2.75	3.97	4.94	6.13
9		Up	0.55	0.89	2.72	3.20	4.22	5.36	6.20
			Storage Temperature 30^{0}C						
10		Hl	0.55	0.91	1.17	1.49	1.47	1.80	2.00
11	30	Bp	0.55	0.98	1.15	1.88	1.87	3.07	4.03
12		Up	0.55	0.98	1.91	3.73	3.84	4.21	4.91
13		Hl	0.55	0.86	1.11	1.53	1.47	1.82	2.02
14	55	Bp	0.55	1.00	1.28	3.36	3.79	4.73	5.55
15		Up	0.55	0.98	1.41	3.43	4.07	5.14	5.97
16		Hl	0.55	0.91	1.18	1.51	1.50	1.96	2.14
17	75	Bp	0.55	1.00	1.93	3.30	4.15	4.90	6.27
18		Up	0.55	1.00	2.92	3.83	4.59	5.35	6.66
			Storage Temperature 35^{0}C						
19		Hl	0.55	0.91	1.11	1.61	1.67	1.86	2.11
20	30	Bp	0.55	0.98	1.21	2.19	2.21	3.35	4.34
21		Up	0.55	1.00	2.82	4.28	4.44	4.69	5.28
22		Hl	0.55	0.92	1.16	1.57	1.54	1.83	2.17
23	55	Bp	0.55	0.95	1.33	3.16	3.13	4.21	5.66
24		Up	0.55	1.00	1.50	3.43	4.32	5.42	6.19
25		Hl	0.55	0.92	1.17	1.58	1.53	1.91	2.21
26	75	Bp	0.55	0.98	2.89	3.75	4.59	5.66	6.61
27		Up	0.55	1.00	3.05	4.46	5.15	6.03	7.22
			Storage Temperature 40^{0}C						
28		Hl	0.55	0.91	1.21	1.92	1.93	2.10	2.41
29	30	Bp	0.55	0.92	3.02	4.47	4.58	5.06	5.88
30		Up	0.55	1.01	3.31	4.58	4.93	5.56	6.23
31		Hl	0.55	1.00	1.36	1.85	1.84	1.88	2.23
32	55	Bp	0.55	0.95	1.50	3.73	4.37	5.55	6.58
33		Up	0.55	1.01	1.67	3.83	4.41	5.70	7.08
34		Hl	0.55	0.95	1.35	1.88	1.79	1.99	2.27
35	75	Bp	0.55	0.98	3.03	4.65	5.46	6.25	7.14
36		Up	0.55	1.00	1.90	4.97	5.85	6.55	7.78

All experimental combinations showed that FFA value linearly increases with storage period. The linear regression of FFA and storage period for different temperature and Rh indicated relationship in view of 'R^2 'value being more than 0.95 and showing low associated error. The following linear mathematical model could explained the behaviour of rise of FFA at different experimental condition for different pack chocolate.

FFA = M S_t + N 4

Where,

FFA = Free fatty acid (per cent oleic acid)

S_t = Storage period, days M and N are coefficients and constant.

M and N are coefficient and constant

The statistical parameters are shown in Table 3.10. R^2 value for linear model varied from 0.954 to 0.997 and the value of standard deviation was in the range of 0.031 to 0.312.

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Effect of Peroxide Value (Meqo$_2$/ kg fat) with Storage Time

Lipid oxidation is the main cause of spoilage and off flavour formation in chocolate. Peroxide value is one major of lipid oxidation Antonio (2003). The peroxide value for chocolate at the start of storage was 0.55 Meqo$_2$/ kg fat which is the safe value for chocolate as reported by Antonio (2003). The table 3 shows the peroxide value (Meqo$_2$/ kg fat) at different relative humidity and temperature during storage period of 130 days for chocolate. The peroxide value of heat sealed laminated chocolate varied from initial value (0.55 MeqO$_2$/ kg fat) to 1.92, 1.95 and 2.20 Meqo$_2$/ kg fat for 30, 55 and 75 per cent relative humidity at 25^{0}C where as for unpacked chocolate peroxide value at the same temperature (25^{0}C) reached up to 4.66, 5.91 and 6.20 Meqo$_2$/ kg fat by the end of 130 days of storage period.

At 40^{0}C heat sealed laminated packaging had peroxide value 2.41, 2.23 and 2.27 Meqo$_2$/ kg fat; for butter paper packing and 5.88, 6.58, 7.14 and 6.23, 7.08 and 7.78 Meqo$_2$/ kg fat for unpacked chocolate at 30, 55 and 75 per cent relative humidity. The increase of peroxide value with storage time, but the value was found less than 10 Meqo$_2$/ kg fat which could be considered at beginning of alteration of fat (Mattisek et al, 1976). In case of heat sealed laminated packing and butter paper packing there was no odder or strange flavour even at the end of 130 days of storage period. The odder and flavour of chocolate influenced by storage period and storage condition, it is conforming the finding of Antonio (2003).

The linear model was attempt to describe the phenomenon of increase of peroxide value (pv) of chocolate with time and the following model was found to correlate the experimental observation satisfactorily :

$Dp = a + bS_t$ 5

Where;

Dp = peroxide value in Meqo$_2$/ kg fat and

S_t = Storage period in days

a and b are constant and coefficient

The coefficients area and b for all chocolate (heat sealed, butter paper packed and unpacked) were obtained through linear regression model. The overall constant, a and b for all experimented chocolates were -0.146 to 0.642 and 0.011 to 0.061. The model could describe the data reasonably well as higher value of 'R^2' value (0.991 to 0.964) for all three packed chocolates. This model was further supported by relatively small associated error.

CONCLUSIONS

The chocolate quality was detected in terms of moisture content, FFA, and PV; these values were at the time of procurement respectively 0.21 Per Cent db, 0.35 per cent Oleic acids, 0.55 MeqO$_2$/Kg, After 130 days of storage the maximum value of moisture content, FFA, PV were respectively 1.74 (per cent db), 1.39 (per cent Oleic acid), 2.27 (MeqO$_2$/Kg Fat), for heat sealed laminated packed chocolate. The results showed that these values were rise with increasing relative humidity, temperature and storage duration. By the above analysis following conclusion could be drawn:

The chocolate stored at temperature 35°C and 40°C and higher relative humidity (>55 per cent) showed higher absorption of moisture.

The heat sealed laminated chocolates absorb less per cent of moisture as compare to butter paper packaging where as the unpacked chocolate is very sensitive to absorb moisture at lower relative humidity also, hence we can conclude the heat sealed laminated packaging is better than other packaging.

The chocolate stored at higher relative humidity and high temperature (≤ 40°C) showed rancid flavour, it may be due to storage at higher temperature and relative humidity chocolate lost its flavour by oxidation of fat and become rancid. FFA and PV values were increased with increasing temperature, Rh and storage duration

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