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RESEARCH ARTICLE

MOISTURE SORPTION CHARACTERISTICS AND STORAGE REGIME OF DEFATTED GRAPE SEEDS FLOUR – ENOLOGICAL BY-PRODUCT

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ABSTRACT

The current scientific research is focused on the moisture equilibrium data (adsorption and desorption) of defatted grape seeds flour at 10, 25 and 40 °C using the static gravimetric method of saturated salt solution and relative humidity from 0.11 to 0.90. The results showed that the sorption capacity decreased when the temperature increases in the conditions of constant water activity. The modified Henderson model describe satisfactorily the sorption isotherms. The MMC was determined using Brunauer-Emmett-Teller (BET) equation that results for adsorption were in the range of 2.61 to 4.81 % and for desorption – from 2.67 to 4.25 %. We were optimized the storage regime up to six-month of the flour in plastic bags at temperature 25 ºC and relative humidity 75 %. No living cells of pathogenic organisms (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp.) or apparent molding were detected. The flour particle size has not changed either.

INTRODUCTION

Modern healthy lifestyle and scientific recommendations for improving the eating habits of consumers was prompted (have the tendency) to use of foods, knows as functional, whose regular use reduces the risk of deepening some chronic diseases or prevent the appearance of others (Calvo *et al*., 2017) (Nowshehri *et al*., 2015). Example of the natural product rich in bioactive compounds is the grape (Teixeira *et al*., 2014). The grape is a berry fruit whose structure is composed of cluster and grains. Each grain is composed of skin, seeds and pulp (the plastic element). The skeleton of the grape is formed by cluster and skins and, together with the seeds form the solids parts (the solid residue) (Pandeliev *et al*., 2010). Furthermore, the grape is the essential ingredient of the wine elaboration (Nogales-Bueno *et al*., 2017). In general, the clusters are from 3 to 7 % of the weight of the grape, skink – from 8 to 20% of the weight of the grapes, the seeds are from 1 to 4, frequently 2 (there are varieties which do not have a seed), and pulp from 70% to 75% of the weight of the grapes (Nogales-Bueno *et al*., 2017). The pulp principally contains water, carbohydrates, organic acids, minerals, nitrogenous and aromatic compounds.

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The seeds have tannins and nitrogenous compounds, which partially pass through the wine during fermentation (Fredes *et al*., 2017), (Abrasheva *et al*., 2009). Due to different grape varieties, climatic and soil characteristics, terroir, agro-technical, technological operation, (i.e. natural and human factors) each lot of retrieved grape seeds after alcoholic fermentation of wine are with variated physico-chemical composition. Approximately, 20% of the grapes are a waste product - include the skins, the seeds and the clusters (Peighambardoust *et al*., 2014*)*. As an oenological sub-product, the grape pomace is used in animal livestock breeding (as a feed additive – because his rich in fibers) (Song *et al*., 2017), food additive, cosmetic and pharmaceutical industries (Kappagoda *et al*., 2017) (Pinna *et al*., 2017). Several scientific studies are proved the beneficial effects of grape seeds, retrieved after alcoholic fermentation of wine, which indicated for antioxidant, antibacterial, antiproliferative, chemoprotective and anti-inflammatory properties on the human organism (Ricci *et al*., 2017), (Klancnik *et al*., 2017), (Ghouila *et al*., 2017), (Cadiz-Gurrea *et al*., 2017), (Hernández *et al*., 2017), (Simonetti *et al*., 2017). In the powdered form, the food ingredient is added as functional additive in bread, pancakes, cereal desserts, noodles, Turkish dry fermented sausage (Sucuk) (Kurt, 2016), fettuccini (Voltaire *et al*., 2014), frankfurters (Özvural and Vural, 2011) and other production (Hoye *et al*., 2011), (Wang *et al*., 2016).

Knowledge of the moisture sorption characteristics (relative humidity and MMC), is related with the optimal conditions of storage regime. This information contributed for preservation of nutritional qualities in the processes such as drying and packaging. The relationships between equilibrium moisture content (M) or (EMC) and water activities (relative air humidity (*Rh*) is showed by experimentally constructed in the condition at a temperature constant. Moisture value corresponding to MMC is an important sorption characteristic, which influence directly on stability of product (Вълодов, 2002; Karel, 1973). MMC can be considered such a critical point of sorption isotherm and with her found have an importance for her storage regime. Conditions (temperature and relative air humidity), where the product is on MMC, can be indicate as optimal for his storage. This were necessitated the identification of Mm for a large number of food products. According to the literature research, Iglesias's and Chirife's analysis are provided Mm of over 50 products (Iglesias and Chirife, 1976). A number of models have been suggested in the literature for the dependence between the equilibrium moisture content (EMC) and the water activity () (Van den Berg and Bruin, 1981). The modified Chung–Pfost, modified Henderson, modified Halsey and modified Oswin equations which incorporate the temperature effect have been proposed by Chen and Morey, (1989). The monolayer moisture content (MMC) for each temperature is calculated by using the Brunauer-Emmett-Teller (BET) equation. The models have been adopted as standard equations by the ASAE for the description of sorption isotherms (ASAE, 2001).

The aims of the present work were to

- characterize the sorption isotherms (adsorption and desorption) at 10, 25 and 40 °C at different water activities (= 0.11 ÷ 0.90) of defatted grape seeds flour (of different grape varieties of Bulgarian origin, namely Mavrud, Cabernet Sauvignon, Syrah, Merlot, Dimyat and Sauvignon Blanc);
- choice an adequate model to describe the sorption isotherms, distribution of residuals and calculation of MMC;
- examine the changes of microbial parameters, granulometric composition and moisture content during 6 months storage of 25 °C and 75 % relative humidity (packed in co-extruded barrier film with copolymer covering for heat sealing) at humidity of product approximately equal of the calculated MMC.

MATERIALS AND METHODS

Materials

Experimental institute located in Parvenets, Bulgaria was delivered defatted grape seeds flour. Grape seeds (of different grape varieties of Bulgarian origin, namely Mavrud, Cabernet Sauvignon, Syrah, Merlot, Dimyat and Sauvignon Blanc) were extracted after alcoholic fermentation of wine as an enological by-product. They were dried under atmospheric conditions and subjected to oil extraction using a screw press. Different grape varieties from Bulgarian grape cultivars were blended in order to obtain sufficient amount of samples for analysis. After de-oiling phase, the grape seeds were milled to a flour. In order to optimize the storage period, the product was packaged in a co-extruded barrier film with copolymer covering for heat sealing designed for food industry, produced by Itaplast "ET - Ilko Tyanevita Plast", Assenovgrad, Bulgaria.

Procedure

The EMC of the defatted grape seeds flour was determined at 10, 25 and 40 º C and = 0.11 ÷ 0.90. The static gravimetric method was applied (Wolf *et al.*, 1985). For the adsorption process, the flour was dehydrated in a desiccator with P_2O_5 at a room temperature for 20 days prior to the beginning of the experiment. The desorption isotherms were determined on samples hydrated in a glass jar over distilled water at a room temperature. Samples of 1 ÷ 0.02 g were weighed in weighing bottles. The weighing bottles were then put in hygrostats with six saturated salt solutions (LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $MgNO_3$, NaBr, NaCl, KCl) used to obtain constant water activity environments (Bell and Labuza, 2000). All of the used salts were of reagent grade. At high water activities (>0.70) crystalline thymol was placed in the hygrostats to prevent microbial spoilage of the flour. The hygrostats were kept in thermostats at 10, 25 and 40 ÷ 0.2ºC. Samples were weighed (balance sensitivity ÷ 0.0001 g) every three days. Equilibrium was ascertained when three consecutive weight measurements showed a difference less than 0.001 g. The moisture content (%) was determined according to AOAC 960.39.

Analysis of data

The following models were used to verify the description of the sorption isotherms:

Modified Chung-Pfost

$$a_w = exp\left[\frac{-A}{t+B}\exp(-CM)\right] \quad (1)$$

Modified Halsey

$$a_w = exp\left[\frac{-\exp(A+Bt)}{M^C}\right] \quad (2)$$

Modified Oswin

$$M = (A+Bt)\left(\frac{a_w}{1-a_w}\right)^C \quad (3)$$

Modified Henderson

$$1 - a_w = exp[-A(t+B)M^C] \quad (4)$$

where:

M is the average moisture content, % d.b.;
is the water activity, decimal;
A, *B* and *C* are coefficients;
t is the temperature, ºC.

A nonlinear, least squares regression program was used to fit the four models to the experimental data (all replications). The suitability of the equations was evaluated and compared using the mean relative error P (%); the standard error of moisture (SEM) and the randomness of residuals (Chen and Morey, 1989):

$$P = \frac{100}{N}\sum\left|\frac{M_i - \hat{M}_i}{M_i}\right| \quad (5)$$

$$SEM = \sqrt{\frac{\sum(M_i - \hat{M}_i)^2}{df}} \quad (6)$$

$$e_i = M_i - \hat{M}_i \quad (7)$$

where:

where:

and are experimentally observed and predicted by the model value of the equilibrium moisture content;

N is the number of data points;
A, *B* and *C* are coefficients.
df is the number of degree of freedom (number of data points minus number of constants in the model).
The monolayer moisture content (MMC) for each temperature is calculated by using the Brunauer-Emmett-Teller (BET) equation (Brunauer *et al.*, 1938) and the experimental data for water activities up to 0.45 (Bell and Labuza, 2000):\

$$M = \frac{M_e C a_w}{(1-a_w)(1-a_w+Ca_w)} \quad (8)$$

Where

M is the MMC, % d.b.;
is the water activity, decimal;
C is the coefficient.

The microbial load

The microbial load of the product was determined during the one-month storage via: Mesophilic aerobic and facultative anaerobic bacteria, according to Bulgarian State Standard (BSS) EN ISO 4833-2:2014;

Yeasts and fungi, according to (BSS) EN ISO 21527-2:2011;
Escherichia coli, according to (BSS) EN ISO 16649-2:2014;
Salmonella spp., according to (BSS) EN ISO 6579:2003;
Coagulase-positive staphylococci, according to (BSS) EN ISO 6888-1:2005.

Flour particle size

Flour particle size was determined with „ProMel LP – 200" sieve analysis equipment. Based on preliminary analysis, the set of sieves was determined as well as their size. The sieving of the sample in the apparatus continues for ten minutes if it amounts to 100 g.

All tests were run in triplicate.

RESULTS AND DISCUSSION

Moisture sorption analysis of defatted grape seeds flour

The obtained mean values of EMC, based on triplicate measurements for the respective water activity and temperature, are presented in Table 1 for adsorption and in Table 2 for desorption. The EMC values increase with an increase in the temperature at constant a_w. The effect on this type of flour is also manifested in other food products high in sugars. The reason for this is the decomposition of sugars after which the flour starts absorbing larger quantities of water. The same effect also applies to the processes of adsorption and desorption. Similar trends for many foods have reported in the literature (Al-Muhtaseb *et al.*, 2002; Durakova and Menkov, 2005). Fig. 1 gives the experimental data obtained after adsorption and desorption at 10^{o}C. The sorption isotherms have an S-shape profile. The hysteresis effect is statistically significant, at a level of significance α=0.05, in the water activity range 0.1÷0.85. The coefficients for the three-parameter modified models, P and SEM values are presented in Table III for adsorption and Table IV for desorption. The coefficients of P and SEM values for the GAB model are given in Table V.

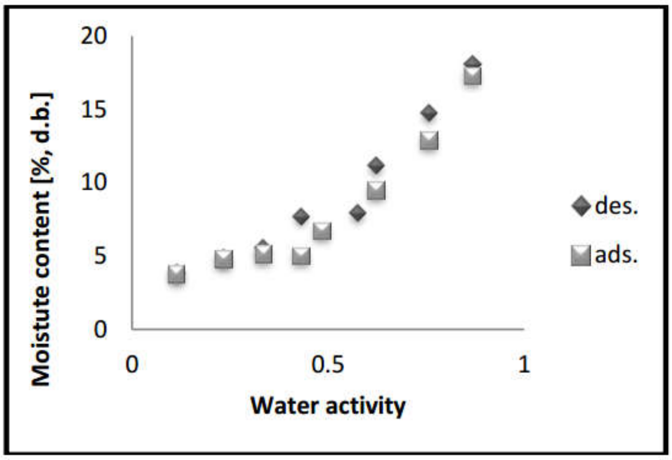

Fig. 1. Comparison of isotherms at 10 °C, Desorption and Adsorption

The results show that the lowest P and SEM values were obtained with the Henderson model. The graphical analysis of the residues demonstrates that the distribution is random for both models, which means that both models are suitable for the description of defatted grape seeds flour sorption isotherms (Fig. 2 and 3). We recommend the Henderson model, because of its lower values of the coefficients.

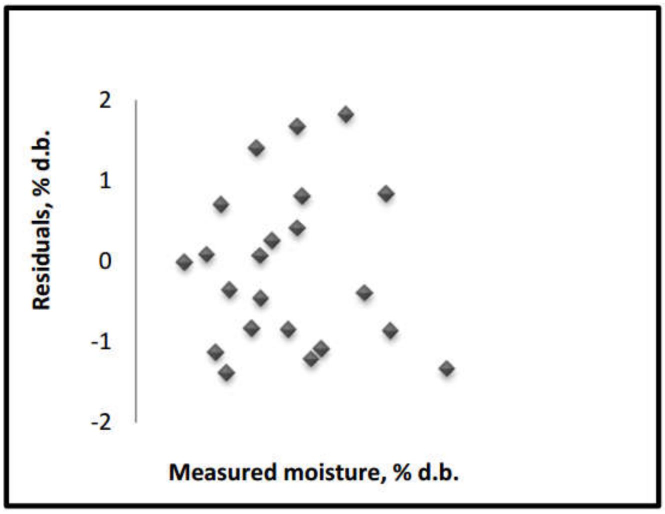

Fig. 2. Plot of residuals fit of modified Henderson model to adsorption data

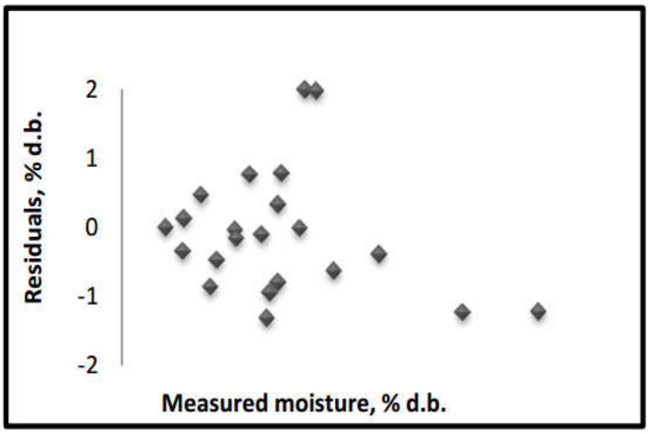

Fig. 3. Plot of residuals fit of modified Henderson model to desorption data

Table 1. Equilibrium moisture content M[a] (% d.b.) of defatted grape seeds flour by adsorption at different water activities () and temperatures *t* (°C)

Sel		10 °C			25 °C			40 °C	
		M[a]	sd[b]		M[a]	sd[b]		M[a]	sd[b]
LiCl	0.113	3.77	0.02	0.113	3.33	0.11	0.112	2.04	0.05
CH_3COOK	0.234	4.81	0.07	0.225	3.91	0.05	0.201	2.95	0.15
$MgCl_2$	0.335	5,16	0.11	0.328	5.18	0.17	0.316	3.54	0.18
K_2CO_3	0.431	5.93	0.08	0.432	5.66	0.12	0.432	6.34	0.04
$MgNO_3$	0.574	6.71	0.03	0.529	6.69	0.06	0.484	7.71	0.21
NaBr	0.622	9.51	0.14	0.576	6.92	0.10	0.532	7.27	0.03
NaCl	0.757	12.91	0.29	0.753	8.74	0.17	0.747	10.40	0.06
KCl	0.868	17.29	0.23	0.843	9.66	0.09	0.823	10.55	0.18

* [a] Mean of three replications
* [b] Standard deviation based on three replications

Table 2. Equilibrium moisture content M[a] (% d.b.) of defatted grape seeds flour by desorption at different water activities () and temperatures *t* (°C)

Sel		10 °C			25 °C			40 °C	
		M[a]	sd[b]		M[a]	sd[b]		M[a]	sd[b]
LiCl	0.113	3.86	0.06	0.113	2.66	0.09	0.112	1.92	0.09
CH_3COOK	0.234	4.92	0.03	0.225	4.14	0.16	0.201	2.70	0.04
$MgCl_2$	0.335	5.57	0.21	0.328	4.98	0.04	0.316	3.44	0.15
K_2CO_3	0.431	7.96	0.27	0.432	6.08	0.22	0.432	6.44	0.43
$MgNO_3$	0.574	7,73	0.29	0.529	6.79	0.32	0.484	6.77	0.14
NaBr	0.622	11.17	0.09	0.576	6.93	0.06	0.532	6.30	0.18
NaCl	0.757	14.78	0.41	0.753	8.43	0.27	0.747	9.21	0.43
KCl	0.868	18.10	0.16	0.843	9.79	0.15	0.823	9.57	0.24

* [a] Mean of three replications
* [b] Standard deviation based on three replications

To calculate the BET monolayer moisture content (MMC), the model (8) is linearly transformed:

$$\frac{a_w}{(1-a_w)M} = P + Qa_w \qquad (9)$$

Based on the coefficients of the linear equation, the MMC for the respective temperature is calculated and the results are presented in Table 5. As a result of the linearization of the BET model and the calculated monomolayer humidity of the defatted grape seeds flour, the shelf life was increased and proven to dry out the test product to a maximum humidity close to the MMC. In previous studies, a three-month storage period during which the "Total number of mesophilic aerobic and facultative anaerobic microorganisms" and "Yeasts and Fungi" were retained from day 1 to day 90 of storage. The microbiological study shows that defatted grape seeds flour with a 9.59 % moisture content packed in plastic bags after three months of storage at 25 ° C and 75 % relative humidity can be used as a nutritional supplement (Bogoeva *et al.*, (2017). In the Table 5, it was presented the results of the microbiological analyzes for a six-month period of storage of defatted grape seeds flour at a humidity of 3.53 % at a temperature of 25 ° C and relative humidity of 75 %. The product under investigation is packaged in plastic bags.

The microbial load

For the aims of the present study, initial humidity was reduced from 9.41 % to 3.53 % (corresponding to already calculated MMC). We were monitored the parameters a storage for 6 months. The flour was subjected to selected microbial and fungal testes, as well. A microbiological analysis was performed at 25 °C and relative humidity of 75 % for 6 months. During a certain period of storage, microbiological parameters such as "Total numbers of mesophilic aerobic and facultative anaerobic bacteria", "*Escherichia coli*", "*Staphylococcus aureus*", "*Salmonella*", " Fungi and yeasts" are determinated.

The results of the storage was monitored on the 1st day, 1st month, 2nd , 3rd , 4th , 5th and 6th months of the storage of defatted grape seeds flour, showed wat Salmonella sp. is not detected and the presence of Escherichia coli and coagulase-positive staphylococci is below the allowable rate on the first day of storage and on the 6th month.

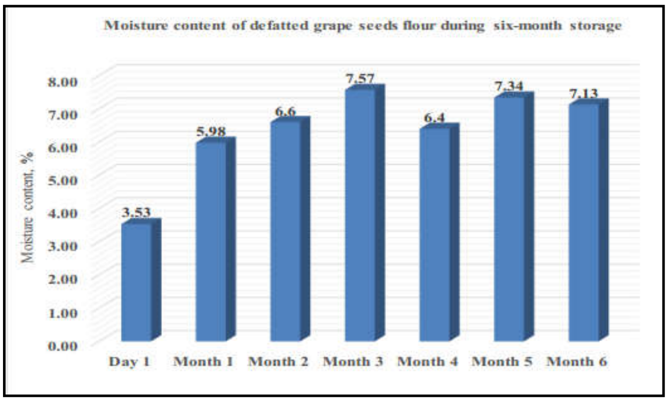

Fig. 4. Moisture content of defatted grape seeds flour during six-month storage

The investigated parameters – "Total numbers of mesophilic aerobic and facultative anaerobic bacteria"" and "Yeast molds" are kept from the first day to 6 months of storage of the defatted meal of grape seeds. Higher microbial cell counts for these two parameters, which are below the permitted levels for this type of food, may be reduced in subsequent heat treatment. The results of the microbiological analyzes show that defatted grape seeds flour natural waste product after alcoholic fermentation in wine elaboration may be stored under the conditions presented in the present experiment for a six-month period without disturbing their nutritional values and microbiological safety. Experimental results show that they can be imported as a food additive and an ingredient in the production of food products. For a six-month storage period, we note that total microbial obsession is maintained.

Table 3. Model coefficients (*A*, *B*, *C*), mean relative error (P, %), standard error of moisture (SEM) and Correlation coefficient (R) for adsorption

Model	*A*	*B*	*C*	P	SEM	R
Oswin	7.70017	-0.04551	0.39767	15.84	1.36	0.940
Halsey	2.639018	-0.005654	1.552695	17.74	1.89	0.952
Henderson	0.000291	95.37602	1.564150	9.56	1.02	0.970
Chung-Pfost	1115.137	0.257156	261.9614	16.08	1.57	0.954

Table 4. Model coefficients (*A*, *B*, *C*), mean relative error (P, %), standard error of moisture (SEM) and Correlation coefficient (R) for desorption

Model	*A*	*B*	*C*	P	SEM	R
Oswin	8.82035	-0.08712	0.39570	13.35	1.33	0.951
Halsey	2.864211	-0.017287	1.500708	17.96	2.24	0.956
Henderson	0.000696	18.61505	1.63518	9.97	1.05	0.975
Chung-Pfost	239.5401	0.255045	36.11601	13.40	1.60	0.960

Table 5. BET monolayer moisture content MMC (% d.b.) of defatted grape seeds flour at several temperatures

t (°C)	Adsorption	Desorption
10	2.76	2.67
25	2.61	2.73
40	4.81	4.25

Table 6 .Microbiological parameters of defatted grape seeds flour for six-month storage at 25 ºC and relative humidity 75 %

Sample/ Day	Total numbers of mesophilic aerobic and facultative anaerobic bacteria, CFU/g	*Escherichia coli*, CFU/g	*Staphylococcus aureus*, CFU/g	*Salmonella sp.* / 25 g	Yeasts, CFU/g	Fungi, CFU/g
Day 1	4.0×10^4	<10	<100	Not detected	<10	3.0×10^2
Month 1	5.0×10^4	<10	<100	Not detected	<10	1.0 x 10
Month 2	2.0×10^4	<10	<100	Not detected	<10	1.0 x 10
Month 3	2.0×10^4	<10	<100	Not detected	<10	1.1×10^2
Month 4	1.5×10^4	<10	<100	Not detected	<10	5.0×10^2
Month 5	3.0×10^4	<10	<100	Not detected	<10	1.0×10^2
Month 6	5.0×10^4	<10	<100	Not detected	<10	1.7×10^2

Table 7 .Granulometric composition of defatted grape seeds flour during six-month storage

№	*Particales size, µm*	*Quantity of break stock, %*			
		1 Day	1 Month	3 Months	6 Months
1	670	10.88	10.35	9.90	9.68
2	560	9.62	9.52	8.53	7.51
3	450	16.54	15.37	14.95	14.20
4	355	56.25	53.92	48.90	47.12
5	280	2.53	2.29	2.85	3.06
6	200	1.55	2.36	6.06	7.81
7	180	0.99	1.74	2.67	3.17
8	150	1.64	2.65	3.58	3.50
9	132	—	1.01	1.28	1.50
10	less than 132	—	0.79	1.28	2.45

No visible molding was observed in the study, nor was the presence of pathogenic microorganisms found.

Flour particle size

As illustrated in table 2, we were monitored the granulometric composition of defatted grape seeds flour during six-month storage period, in the selected conditions – temperature 25 °C and relative humidity of 75 %. The changes of the moisture content values are presented in table 3, as well. The results shows in table 2 are presented the percentage distribution of the quantity of break stock is similar for particles size over 355 µm (from 47.12 % to 56.25 %). During the storage, there is also increase in the amount of smallest-size fractions below 180 µm. The majority of the fractions are distributed between 650 and 355 µm. The differences in results presented in table 2 are corresponded to measured humidity in the same time. We believe that the distribution of flour particles depends to a great extent on the duration of storage, not only on the relative moisture of flour (only for the given moisture range). We defined that the product is suitable for incorporation as a functional ingredient to base flour whose size is over 180 µm. According to our results, we were reported no considerable differences in the granulometric composition of defatted grape seeds flour. During the storage period, we find a change in moisture content results. In the first three months, the humidity of the sample gradually increase from 3.53 % to 7.57 %. After three-month, the results are in the range from 6.40 % to 7.34 %, which we believe is due to the packaging. In the present study, the maximum values of the moisture content for six-month storage is 7.34 %.

Conclusion

- The sorption capacity of defatted grape seeds flour decrease with an increase in temperature at constant water.

- The modified Henderson model is suitable for describing the relationships between the equilibrium moisture content, the water activity and the temperature of the defatted grape seeds flour.
- According to sorption isotherms obtained for 10, 25 and 40 °C, MMC is calculated with BET equation.
- During the six-month storage of the defatted grape seeds flour in plastic bags (at temperature 25 °C and relative humidity 75 %), we were not detected living cells of pathogenic organisms or apparent molding.
- The percentage distribution of flour was not change considerably.
- According to reported results, this flour is available for consummation after 6 months storage period for the conditions of the present experiment.

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