Journal of Agriculture and Food Research 2 (2020) 100026

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.journals.elsevier.com/journal-of-agriculture-and-food-research/

Sorption characteristics of full-fatted grape seeds flour of Bulgarian origin

Adelina L. Bogoeva [a,*], Albena G. Durakova [b]

[a] Institute of Food Preservation and Quality - Plovdiv, Department of Food Technology, 154 Vasil Aprilov Blvd, 4003, Plovdiv, Bulgaria
[b] University of Food Technologies (UFT) – Plovdiv, Department of Process Engineering, 26 Maritsa Blvd, BG-4002, Plovdiv, Bulgaria

ARTICLE INFO

Keywords:
Grape seeds
Flour
Antioxidant activity
Sorption isotherms
Monolayer moisture content
Storage

ABSTRACT

We were experimentally obtained the sorption characteristics of full-fatted grape seeds flour (FGSF) of different grape varieties locally grown in Bulgaria. The scientific research was investigated at temperature - 10 °C, 25 °C and 40 °C and water activity within the range from 11% to 90%. We were selected the modified Oswin and Chung-Pfost models suitable for describing the adsorption and desorption isotherms. Monolayer moisture content of FGSF is calculated – from 2.48% to 4.59%. Antioxidant capacity was proven through DPPH (578.42 ± 23.06 mM TE/g extract), ABTS (1352.12 ± 144.31 mM TE/g extract), FRAP (966.45 ± 127.31 mM TE/g extract) and CUPRAC (1789.44 ± 180.11 mM TE/g extract). The values are also shown in mM TE/g flour (578.42 ± 23.06 mM TE/g flour); (1352.12 ± 144.31 mM TE/g flour); (966.45 ± 127.31 mM TE/g flour) and (1789.44 ± 180.11 mM TE/g flour), respectively. Furthermore, we evaluated the changes in moisture content, microbiological load and particle size distribution during short-term storage study for a period of three months. FGSF were packed in plastic bags under the conditions - temperature 18 °C÷25 °C and relative air humidity 45% ÷ 55%.

1. Introduction

The consumption of grapes and grapes products was reported to have positive effects on human health. Grapes are a good and a natural source of essential nutrients for the organism of humans [1]. Scientific studies confirmed that grapes are a good healthy alternative substitute for synthetic antioxidant agents due to a higher reduction capacity compared to Vitamin E proven by the DPPH and ABTS methods [2]. It has been proven that the fruit has antimicrobial compounds that can inhibit some diseases [3]. During the process of winemaking, large quantities of waste are generated, approximately 20% [4,5]. Grape seeds, including other solid parts of the grape, constitute a substantial waste material. The technological usage of grape seeds in the food industry is a solution to both ecological and economic problems [6,7]. The essential aim at utilization of excess waste and avoidance of undesirable contamination gives additional benefits from applications of grape seeds retrieved after alcoholic fermentation [8]. Furthermore, grape seeds are major source of lipids and are frequently used for animals feed [9,10]. Grape seeds are used in the pharmaceutical and cosmetic industries as well [11]. According to Ghouila et al. [12], the worldwide trend is to search natural sources of antioxidants (from fruits, vegetables and plants) fighting against chronic degenerative diseases. Numerous scientific publication confirmed that grape seeds are rich in bioactive compounds including antioxidants. They are added to foods as a functional nutritional supplement [13,14]. According to Abdrabba and Hussein [4] the highest antioxidant activity reported in grape seeds compared to skins and pulp of the grape. Grape seeds in different forms - flours (full- and de-fatted) and flakes, facilitates their incorporation into various food products. Grape seeds flakes contain approximately the same composition of fat as nutritional values and antioxidants [15,16]. Grape seeds exhibit antidiabetic, antibacterial, anti-cancer, anti-inflammatory, aromatic and antifungal properties. Furthermore, they contain dietary fiber, polymeric flavan 3-ols, oleic, linolenic, palmitic and stearic acids [17–19].

Knowledge of the moisture sorption characteristics (relative humidity and MMC), is related to the optimal conditions of storage regime and it can contribute to the preservation of nutritional qualities and technological properties [20–22].

Moisture value corresponding to MMC is an important sorption characteristic, which influences directly the stability of the product [23–25]. Conditions (temperature and relative air humidity) where the product is with a certain MMC can be indicated as optimal for its storage. Iglesias's and Chirife's [26] analysis provided data for MMC of over 50 products.

The objectives of the present analysis were to:

* Corresponding author.
E-mail addresses: adelina.bogoeva@abv.bg (A.L. Bogoeva), aldurakova@abv.bg (A.G. Durakova).

https://doi.org/10.1016/j.jafr.2020.100026
Received 7 November 2019; Received in revised form 29 January 2020; Accepted 30 January 2020

(1) characterize the sorption isotherms at 10, 25 and 40 °C at different water activities ($a_w = 0.11 \div 0.90$) of full-fatted grape seeds flour (of different grape varieties of Bulgarian origin, namely Mavrud, Cabernet Sauvignon, Syrah, Merlot, Dimyat and Sauvignon Blanc);
(2) Adequate description of the obtained sorption isotherms through modified model, distribution of residuals and calculation of MMC;
(3) determine the antioxidant activity of powdered full-fatted grape seeds;
(4) examine the changes of microbial parameters, particle size distribution and moisture content during short-term storage at three months at 18 °C ÷ 25 °C and at 45% ÷ 55% relative air humidity (packed in co-extruded barrier film with copolymer covering for heat sealing) at moisture content of product approximately equal to the calculated MMC.

2. Materials and methods

2.1. Materials

An experimental institute located in Parvenets, Bulgaria delivered powdered full-fatted grape seeds (of different grape varieties of Bulgarian origin, namely Mavrud, Cabernet Sauvignon, Syrah, Merlot, Dimyat, Sauvignon Blanc). Grape seeds were extracted after alcoholic fermentation of wine as a sub - product. They were dried under atmospheric conditions and milled to obtain a powdered product. Grape seeds were full fat and their high fat content (20.05%) was confirmed by Soxhlet extractor at the Department of Technology of grain, fodder, bread and confectionery products at the University of Food Technologies, Plovdiv, Bulgaria.

For the aims of the storage study, 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.

2.2. Procedure

A static gravimetric method of the sorption isotherms obtained was used in the present work, recommended by Project COST 90 [27] and upgraded by Bell & Labuza, (2000) [28]. Saturated salt solutions (LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $MgNO_3$, NaBr, NaCl, KCl) assure the water activity over them surface within the range from 11% to 90%. Supported temperatures in thermostats are 10 °C, 25 °C and 40 ± 0.2 °C [28]. After EMC (equilibrium moisture content) was reached (20–30 days) the moisture content of the product was determined by drying method according to AOAC 960.39 [29]. Method description is detailed by Durakova et al., 2013 and Durakova et al., 2017 were used for the studies [30, 31].

2.3. Analysis of data

The following three-parameter models of modified Chung-Pfost, modified Halsey, modified Oswin, modified Henderson recommended by American Society of Agricultural Engineers (ASAE), (2002) are selected to verify the description of the sorption isotherms obtained [32]:

2.4. 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

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

Where:

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

On the base of obtained experimental data the different modified models were surveyed through a nonlinear least-squares regression program. Lomauro et al., (1985) consider as an objective criterion for determining model fit is the average relative error P, % [33]. The same analysis concludes that if P ≤ 10% the model can be considered fit. Chen & Morey, (1989) propose to compare the suitability of the models use three criteria: the mean relative error P (%); the standard error of moisture (SEM) and the randomness of residuals [34]. These criteria has been adopted until today [30,31,35]:

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

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

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

Where:

M_i and $\hat{M}_i$ 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).

According to literature review, the monolayer moisture content (MMC) is determined through the calculation of Brunauer-Emmett-Teller (BET) equation. Our obtained results include water activities up to 0.45 for three temperatures – 10 °C, 25 °C and 40 °C [36]:

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

Where.

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

2.5. Methods

2.5.1. Antioxidant activity

The sample of full-fatted grape seeds flour was triple extracted with 10 ml 70% ethanol. The obtained 70% ethanol extract was subjected to investigation for antioxidant capacity with DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azobis (3) -ethylbenzothiazoline-6), FRAP (ferric reducing antioxidant power) and CUPRAC (cupric reducing antioxidant capacity). These four methods are based on different mechanisms

Table 1
Antioxidant activity of 70% ethanol extract of full-fatted grape seeds flour expressed as mM TE/g extract and mM TE/g flour.

METHODS	mM TE/g extract	mM TE/g flour
DPPH	578.42 ± 23.06	72.61 ± 1.78
ABTS	1352.12 ± 144.31	104.21 ± 11.12
FRAP	966.45 ± 127.31	74.48 ± 9.81
CUPRAC	1789.44 ± 180.11	137.91 ± 13.88

action and reaction conditions and confirmed the presence of antioxidant activity [37]. Full description of procedure is detailed in an article by Bogoeva et al., 2017 [16].

2.5.2. The microbial load

The microbial load of the product was determined during the three-month storage via [38]:

Mesophilic aerobic and facultative anaerobic bacteria, according to Bulgarian State Standard (BSS EN ISO 4833–1, 2013) [39]; Yeasts and fungi, according to BSS EN ISO 21527–2, 2011 [40]; *Escherichia coli*, according to BSS EN ISO 16649–2, 2014 [41]; *Salmonella* spp., according to BSS EN ISO 6579–1, 2017 [42]; Coagulase-positive staphylococci, according to BSS EN ISO 6888–1, 2005/A1:2005 [43].

2.5.3. Particle size distribution and moisture content

We were put 100 g of sample on sieves with different size using “ProMel LP – 200” apparatus. The fraction (K) was determined according to procedure described by Bogoeva et al., 2017 [16].

2.5.4. The moisture content (%) was determined according to AOAC, 1990 [29]

All tests were run in triplicate. Data presented are mean values and standard deviations.

3. Results and discussion

3.1. Antioxidant activity

An extract of 77.1 mg (7.71%) was obtained after extraction of 1 g of full-fatted grape seeds flour. The obtained data (mean ± standard deviation) are presented in Table 1 expressed as mM TE/g extract of full-fatted grape seeds flour and as mM TE/g full-fatted grape seeds flour.

According to these values, the analyzed extract of full-fatted grape seeds flour possesses antioxidant activity for all of the tested methods. There was no information in the scientific studies about antioxidant activity of full-fatted flour from Bulgarian varieties of grape seeds. Bogoeva et al., (2017) [16] is reported similar data for defatted grape seeds flour retrieved after alcoholic fermentation in wine elaboration. The results obtained from other authors about the antioxidant activity of grape seeds flour are significantly lower than our results for the Bulgarian full-fatted grape seeds flour. In a study by Binzer et al. [44], the results obtained by the DPPH method for 70% ethanolic extract of grape seeds flour of different varieties (Chardonnay, Concord, Norton, Ruby Red, White) varied between 0.5 mM TE/g flour - 7.0 mM TE/g flour. Lutterodt al. [45], also reported significantly lower antioxidant capacity of seeds flours of varieties Muscadine, Concord, Ruby Red, Chardonnay, Soybean defined by DPPH method (11.8 mM TE/g flour - 15.0 mM TE/g flour). The antioxidants composition of grape seeds depends largely on a heat treatment according to Kim et al., [46]. Probably these differences in the results were due to the varietal and climatic conditions as well as the differences in the oenological practice in wine production and powder technology.

3.2. Moisture sorption analysis of full-fatted grape seeds flour

In Table 2 and Table 3 are showed the obtained results for the equilibrium moisture content (EMC) of Bulgarian grape seeds flour, respectively for adsorption and desorption process under the conditions of the experiment (t = 10 °C, 25 °C and 40 °C and $a_w = 0.1 \div 0.9$). The calculated initial moisture content for the adsorption process is 3.36% and for

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

Sel	10 °C			25 °C			40 °C		
	a_w	M[a]	sd[b]	a_w	M[a]	sd[b]	a_w	M[a]	sd[b]
LiCl	***0.113***	4.09	0.09	***0.113***	2.89	0.06	***0.112***	2.75	0.04
CH_3COOK	***0.234***	5.03	0.12	***0.225***	4.58	0.35	***0.201***	4.23	0.20
$MgCl_2$	***0.335***	5.32	0.02	***0.328***	4.97	0.08	***0.316***	4.84	0.21
K_2CO_3	***0.431***	6.12	0.01	***0.432***	5.75	0.08	***0.432***	5.18	0.03
$MgNO_3$	***0.574***	8.38	0.14	***0.529***	6.68	0.09	***0.484***	5.98	0.21
NaBr	***0.622***	8.61	0.09	***0.576***	7.11	0.20	***0.532***	6.49	0.30
NaCl	***0.757***	14.06	0.33	***0.753***	8.12	0.20	***0.747***	8.86	0.07
KCl	***0.868***	18.41	0.23	***0.843***	9.51	0.09	***0.823***	8.99	0.20

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

Table 3
Equilibrium moisture content M[a] (% d.b.) of full-fatted grape seeds flour by desorption at different water activities (a_w) and temperatures *t* (°C).

Sel	10 °C			25 °C			40 °C		
	a_w	M[a]	sd[b]	a_w	M[a]	sd[b]	a_w	M[a]	sd[b]
LiCl	***0.113***	3.68	0.07	***0.113***	3.41	0.01	***0.112***	2.82	0.10
CH_3COOK	***0.234***	5.61	0.25	***0.225***	4.26	0.31	***0.201***	3.99	0.24
$MgCl_2$	***0.335***	5.86	0.10	***0.328***	5.81	0.07	***0.316***	4.99	0.07
K_2CO_3	***0.431***	7.78	0.18	***0.432***	5.96	0.18	***0.432***	5.79	0.13
$MgNO_3$	***0.574***	8.12	0.08	***0.529***	6.67	0.21	***0.484***	6.04	0.24
NaBr	***0.622***	12.16	0.14	***0.576***	7.93	0.34	***0.532***	7.63	0.28
NaCl	***0.757***	14.95	0.05	***0.753***	9.09	0.22	***0.747***	8.16	0.10
KCl	***0.868***	17.66	0.35	***0.843***	11.23	0.09	***0.823***	9.41	0.16

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

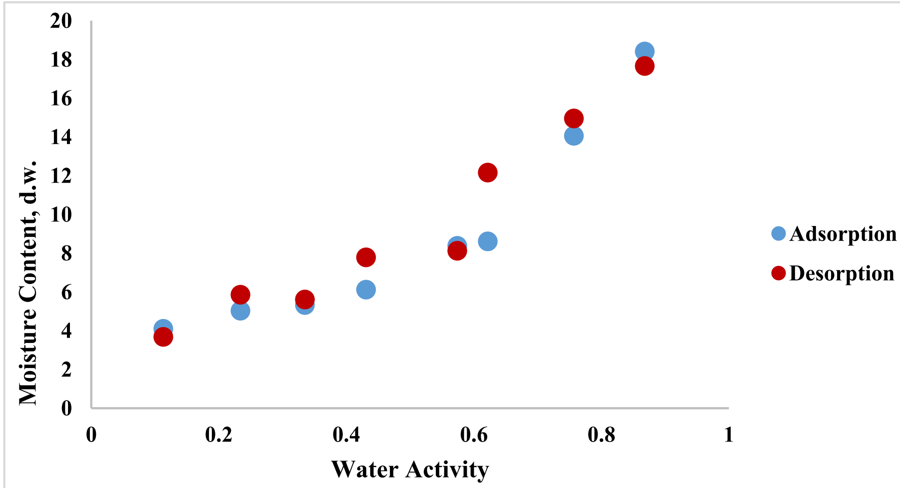


Fig. 1. Comparison of isotherms at 10 °C, Desorption and Adsorption.

Table 4
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	8.81530	−0.08904	0.37761	13.19	1.28	0.946
Halsey	3.77427	−0.01195	2.07484	10.55	1.49	0.976
Henderson	0.000214	2.03567	2.54942	14.26	1.63	0.911
Chung-Pfost	**547.982**	**0.33870**	**61.6750**	**9.57**	**1.93**	**0.968**

Table 5
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	9.68658	−0.10298	0.35869	10.06	1.11	0.962
Halsey	3.46661	−0.01567	1.79188	13.08	1.57	0.969
Henderson	0.000153	2.879898	0.18056	17.33	2.21	0.949
Chung-Pfost	**305.881**	**0.27126**	**39.46184**	**12.98**	**1.58**	**0.955**

the process desorption - 19.25%. EMC values under the selected storage conditions (relative air humidity - 45%–55% and temperature - 18 °C–25 °C) are within the range from 5.75% to 8.38%. The results show that the EMC varied from 3% to 18% over the whole period of the study, with the increase of the temperatures the EMC decreases at a constant a_w. Based on the literature review, similar conclusions are reported for many foods [47,48].

Fig. 1 compares the equilibrium isotherms obtained for the two processes - adsorption and desorption at 10 °C.

According to Brunauer et al., 1940 [49] our graphical analysis is a confirmatory *S*-shape profile (type II). Similar results were obtained for the other two studied temperatures.

In Table 4 and Table 5 are showed the calculated results of coefficients of the three-parameter modified models and the corresponding mean relative error (P, %), standard error of moisture (SEM) and correlation coefficient (R).

Adsorption lowest mean relative error (P) value is reported for the modified Chung-Pfost model and standard error of moisture (SEM) - the

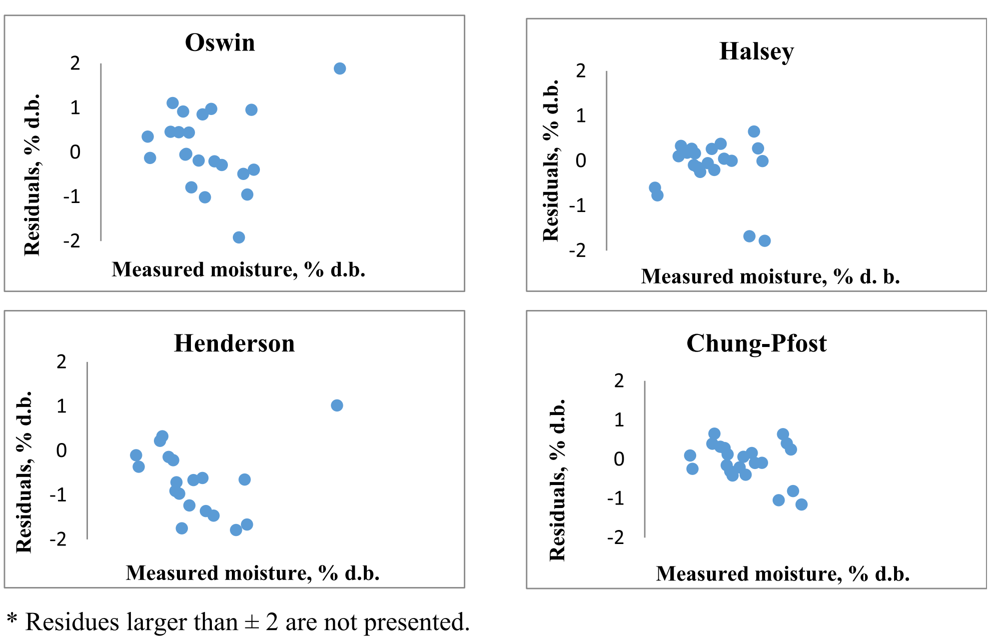
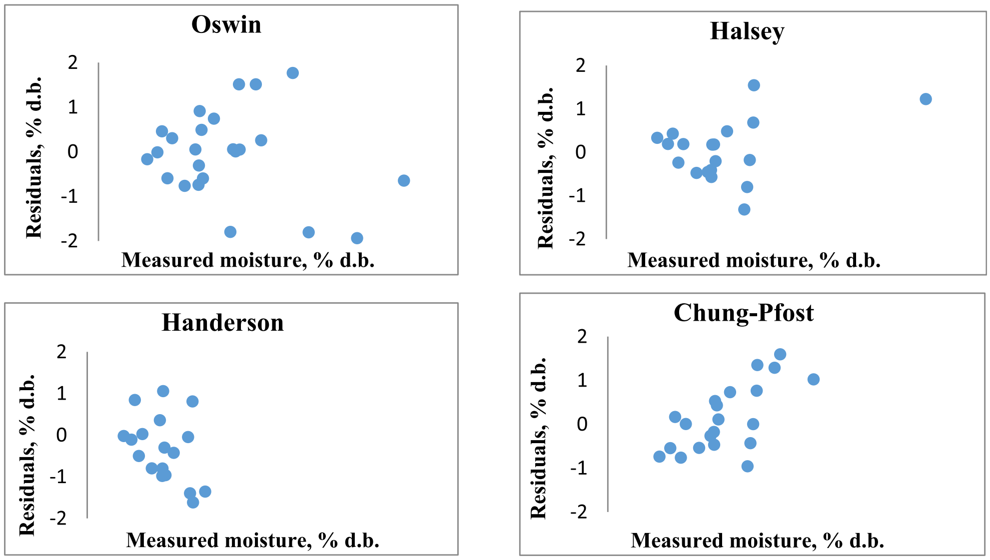


Fig. 2. Plot of residuals fit for modified Oswin, Halsey, Henderson and Chung-Pfost model to adsorption data.



* Residues larger than ± 2 are not presented.

Fig. 3. Plot of residuals fit for modified Oswin, Halsey, Henderson and Chung-Pfost model to desorption data.

modified Oswin model. We recommended the two models to describe isotherms. For desorption isotherms we offered a modified Oswin model that meets commonly accepted standards (lowest P and SEM values).

The graphical analysis of residue distribution presented in Fig. 2 and Fig. 3(for adsorption and desorption respectively) confirms the suitability of the modified Oswin model which for both processes is of a random nature. On the basis of obtained results we can offer (recommended) both modified Chung-Pfost and Oswin models which we consider satisfactory to describe the sorption isotherms of Bulgarian full-fatted grape seeds flour.

To calculate the BET monolayer moisture content (MMC) the model (8) is linearly transformed in Fig. 4 for adsorption process and Fig.5 for desorption process:

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

The values of MMC presented in Table 6, based on the coefficient of linear equation (see Fig.4 and Fig. 5) , were varied within the range from 2.48% to 4.59% d.b. The hysteresis effect is present, the desorption results are higher than the adsorption results of 0.2% d.b. to 1.2% d.b. The lowest values for the flour are observed at a temperature of 25 °C.

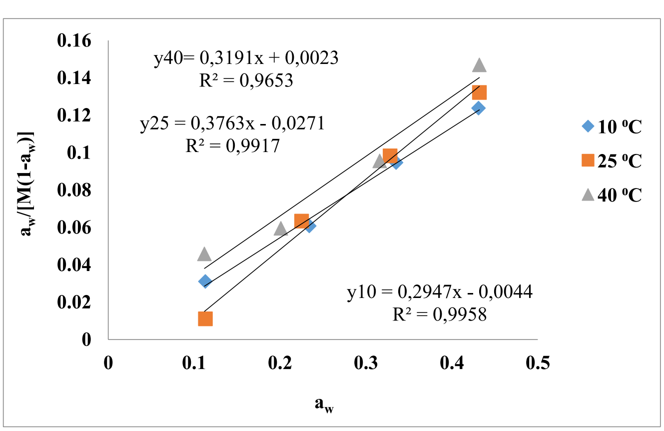


Fig. 4. Full-fatted grape seeds flour: Linearization of the BET model for different adsorption temperatures.

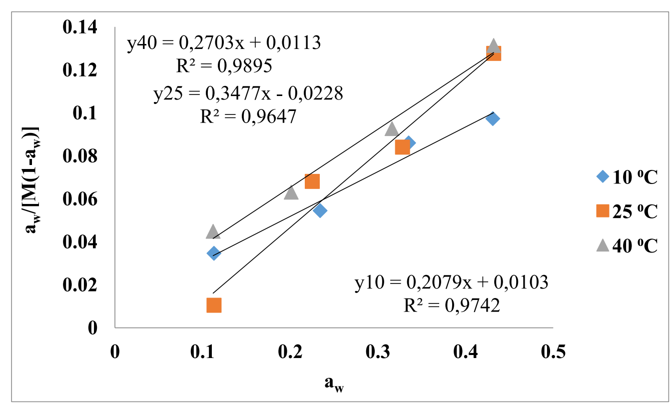


Fig. 5. Full-fatted grape seeds flour: Linearization of the BET model for different desorption temperatures.

Based on the linearization of the BET model (see Fig. 4 and Fig. 5), the monolayer moisture content of the Bulgarian grape seeds flour was calculated. The obtained values assure an extended shelf life of the product, i.e. when the moisture is within the range of the corresponding MMC.

3.3. Tracking the changes in parameters - moisture content, microbiological load and granulometric composition at three months

Moisture content is a significant parameter influencing the quality of food products. In case of powdery products its growth leads to regrouping of the powdered particles (agglomeration) which affects both the

Table 6
BET monolayer moisture content MMC (% d.b.) of full-fatted grape seeds flour at several temperatures.

t (°C)	Adsorption	Desorption
10	3.34	4.59
25	2.48	2.70
40	3.11	3.55

Table 7
Moisture content changes of full-fatted grape seeds flour during short-term storage for three month.

Day/Month	Day 1	Month 1	Month 2	Month 3
Moisture content, %	4.1	5.8	5.8	6.0

Table 8
Particle size distribution during short-term storage of full-fatted grape seeds powder.

Particles size class, μm		Quantity of break stock, %	
Day/Month	Day 1	Month 1	Month 3
1000/600	7.4	7.2	7.9
600/400	49.5	70.6	79.4
400/200	43.1	22.2	12.7
200/	–	–	–

granulometric composition and the microbiological load.

The aim of the study is to trace the change in moisture content (MC) of the studied new products for the Bulgarian market during short-term storage. MC changes are tracked for samples taken to a MC corresponding to the previously calculated MMC of 2.48%–4.59%. Major attention is paid to the change in MC of the products studied and the time to reach their equilibrium moisture content for the conditions of the experiment.

During the analyses the products were packaged in a co-extruded barrier foil with a copolymer coating for heat treatment intended for the food industry.

Experiment conditions correspond to those under which flour products are stored in storehouses - a temperature within the range from 18 °C to 25 °C and a relative air humidity of 45%–55%. The samples were dried over P_2O_5 to attain the moisture content corresponding to the calculated MMC.

The results do not claim sufficiency with a view to setting regimes and storage deadlines but we believe they are a good basis for developing in-depth research in this area.

The change in MC values at short-term storage at three months is presented in Table 7. The duration of the reported period considered the high value of fat in the product - 20.05%.

MMC values varied from 4.1% to 6.0% during three-storage. A noticeable change is observed from the first day to the first month with the increase being 1.7% while over the remaining equal reporting periods the values were approximately the same.

The analyses continue with the determination of the granulometric composition of full-grape seeds flour presented in Table 8.

The degree of milling of grape seeds to full-fat flour is little as their fat content is high and makes the process difficult. About 80%–90% of the fractions have a particle size of 200 μm–600 μm. Most likely (Probably), the increased amount of fat causes agglomeration of particles of the 400/200 class fraction to the 600/400 class fraction in the storage process. The low moisture content of the product corresponding to MMC is the basis of an increase in the 600/400 fraction by about 21.1% in the first month. In the experimental short-term storage the basic amount of break stock was of 600 μm ÷ 400 μm as their ratio changes over the whole period. On the first day the quantity is 49.5%, for the first month there is an increase - 70.6% and in the third month for the same fraction the quantity increases to 79.4%. From the presented data we confirm the appearance of a partial agglomeration. According to the data presented we can note that the particles size distribution depends on the MC which depends directly on which season storage takes place. Based on the obtained data base we consider that full-fatted grape seeds can be included as a functional ingredient in a base whole flour.

3.3.1. *The microbial load*

During the storage the microbiological parameters for *Escherichia coli*, *Salmonella* sp., coagulase-positive staphylococci, total numbers of mesophilic aerobic and facultative anaerobic bacteria, yeasts, and fungi were tracked.

The change in microbiological parameters during short-term storage is presented in Table 9.

The presence of pathogenic micro-organisms, in particular *Escherichia coli*, coagulase-positive staphylococci, total number of mesophilic aerobic and facultative anaerobic microorganisms, yeasts and yeasts are below the admissible norms and *Salmonella* sp. is not detected for the entire period of the short-term storage. The results demonstrate that the microbiological purity of the product is maintained from the first to the last day of storage. There is a visible decrease in growth of molds when stored with MMC relative to the storage at the product's original moisture content. This gives us reason to believe that the product can be stored for experimental conditions without disturbing its microbiological flora.

4. Conclusions

1. The antioxidant activity of Bulgarian grape seeds flour is confirmed via DPPH, ABTS, FRAP and CUPRAC (578.42 ± 23.06 mM TE/g flour); (1352.12 ± 144.31 mM TE/g flour); (966.45 ± 127.31 mM TE/g flour) and (1789.44 ± 180.11 mM TE/g flour), respectively.
2. The sorption capacity of full-fatted grape seeds flour (FGSF) of different grape varieties of Bulgarian origin, namely Mavrud, Cabernet Sauvignon, Syrah, Merlot, Dimyat and Sauvignon Blanc decreases with increasing the temperature for both adsorption and desorption process at each water activity condition.
3. The modified Oswin and the modified Chung-Pfost models are suitable for describing the obtained sorption isotherms of the Bulgarian full-fatted grape seeds flour.
4. According to linearization of BET equation, the monolayer moisture content is calculated at 10 °C, 25 °C and 40 °C (for adsorption – from 2.48% to 3.34% and for desorption – from 2.70% to 4.59%).
5. During three months storage at MMC the reached moisture value is within the range of equilibrium moisture content (from 5.75% to 8.38%).

Table 9
Microbiological parameters changes of full-fatted grape seeds flour during three-month short-term storage.

Sample/Storage period (days)	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	2.0×10^5	<10	<100	Not detected	<10	5.0×10^3
Month 1	7.2×10^5	<10	<100	Not detected	<10	5.0×10^3
Month 2	6.3×10^5	<10	<100	Not detected	<10	4.7×10^3
Month 3	5.5×10^4	<10	<100	Not detected	<10	4.5×10^3

Acknowledgments

This study was conducted with the kind support of the UNESCO Chair "Culture & Traditions of Wines" at Institute of Vine and Wine (IUVV – Institut « Jules Guyot»), University of Burgundy, Dijon, France.

References

[1] S.S. Percival, Grape consumption supports immunity in animals and humans, J. Nutr. 139 (2009) 1801S–1805S.
[2] G.N. Yonguc, Y. Dodurga, E. Adiguzel, G. Gundogdu, V. Kucukatay, S. Ozbal, I. Yilmaz, U. Cankurt, Y. Yilmaz, I. Akdogan, Grape seed extract has superior beneficial effects than vitamin E on oxidative stress and apoptosis in the hippocampus of streptozotocin induced diabetic rats, Gene 555 (2015) 119–126.
[3] C.D. Wu, Grape products and oral health, J. Nutr. 139 (2009) 1818S–1823S.
[4] S. Abdrabba, S. Hussein, Chemical composition of pulp, seed and peel of red grape from Libya, Glob. J. Sci. Res. J. 3 (2015) 6–11.
[5] J. García-Lomillo, M.L. González-SanJosé, Applications of wine pomace in the food industry: approaches and functions, Compr. Rev. Food Sci. Food Saf. 16 (2017) 3–22.
[6] D. Apaydin, A.S. Demirci, U. Gecgel, Effect of gamma irradiation on biochemical properties of grape seeds, J. Am. Oil Chem. Soc. 94 (2017) 57–67.
[7] J. Burton, Making sense of waste flakes: new methods for investigating the technology and economics behind chipped stone assemblages, J. Archaeol. Sci. 7 (1980) 131–148.
[8] Y. Song, L. Zheng, X. Zhang, Kinetics model for supercritical fluid extraction with variable mass transport, Int. J. Heat Mass Tran. 112 (2017) 876–881.
[9] O. Famuyiwa, C. Ough, Grape pomace: possibilities as animal feed, Am. J. Enol. Vitic. 33 (1982) 44–46.
[10] D.L. Bath, Feed By-Products and Their Utilization by Ruminants, Upgrading Residues and By-Products for Animals, CRC Press, 2018, pp. 1–16.
[11] G. Simonetti, F.D. D'auria, N. Mulinacci, M. Innocenti, D. Antonacci, L. Angiolella, A.R. Santamaria, A. Valletta, L. Donati, G. Pasqua, Anti-dermatophyte and anti-malassezia activity of extracts rich in polymeric flavan-3-ols obtained from vitis vinifera seeds, Phytother Res. 31 (2017) 124–131.
[12] Z. Ghouila, S. Laurent, S. Boutry, L. Vander Elst, F. Nateche, R. Muller, A. Baaliouamer, Antioxidant, antibacterial and cell toxicity effects of polyphenols Fromahmeur bouamer grape seed extracts, J. Fund. Appl. Sci. 9 (2017) 392–420.
[13] M.J. Schuster, X. Wang, T. Hawkins, J.E. Painter, A Comprehensive review of raisins and raisin components and their relationship to human health, J. Nutr. Health 50 (2017) 203–216.
[14] C. Beres, F.F. Simas-Tosin, I. Cabezudo, S.P. Freitas, M. Iacomini, C. Mellinger-Silva, L.M. Cabral, Antioxidant dietary fibre recovery from Brazilian Pinot noir grape pomace, Food Chem. 201 (2016) 145–152.
[15] A.G. Durakova, A.L. Bogoeva, A.I. Pavlov, K.T. Dinkov, R.Z. Vrancheva, V.B. Yanakieva, Antioxidant activity and storage regime of grape seeds flakes – a waste product in wine elaboration, Bulgarian J. Agricult. Sci. 24 (2018) 503–508.
[16] A.L. Bogoeva, A.G. Durakova, A.I. Pavlov, V.B. Yanakieva, R.Z. Vrancheva, B.V. Bozadzhiev, K.B. Choroleeva, Antioxidant activity and storage regime of defatted grape seeds flour, Wine Stud. 6 (2017) 1–6.
[17] N.G. Baydar, G. Özkan, O. Sağdiç, Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts, Food Contr. 15 (2004) 335–339.
[18] A. Ricci, G.P. Parpinello, A.S. Palma, N. Teslić, C. Brilli, A. Pizzi, A. Versari, Analytical profiling of food-grade extracts from grape (*Vitis vinifera* sp.) seeds and skins, green tea (*Camellia sinensis*) leaves and Limousin oak (*Quercus robur*) heartwood using MALDI-TOF-MS, ICP-MS and spectrophotometric methods, J. Food Compos. Anal. 59 (2017) 95–104.
[19] M.M. Hernández, S. Song, C.M. Menéndez, Influence of genetic and vintage factors in flavan-3-ol composition of grape seeds of a segregating *Vitis vinifera* population, J. Sci. Food Agric. 97 (2017) 236–243.
[20] Z. Berk, Food Process Engineering and Technology, Academic press, 2018, 0128120541.
[21] M.C. Bourne, Effects of Water Activity on Textural Properties of Food Water Activity Routledge, 2017, pp. 75–99.
[22] F.E. Vasile, M.A. Judis, M.F. Mazzobre, Impact of Prosopis alba exudate gum on sorption properties and physical stability of fish oil alginate beads prepared by ionic gelation, Food Chem. 250 (2018) 75–82.
[23] J. Troller, Water Activity and Food, Elsevier, 2012, 032315901X.
[24] A.L. Decagon, Fundamentals of Moisture Sorption Isotherms. Application Note, Decagon Devices, Pullman, WA Google Scholar, 2011.
[25] S. Kaya, T. Kahyaoglu, Thermodynamic properties and sorption equilibrium of pestil (grape leather), J. Food Eng. 71 (2) (2005) 200–207.
[26] H. Iglesias, J. Chirife, Prediction of the effect of temperature on water sorption isotherms of food material, Int. J. Food Sci. Technol. 11 (2) (1976) 109–116.
[27] W. Wolf, W.E.L. Spiess, G. Jung, Standar-dization of isotherm measurements (COST-Project 90 and 90 bis), in: D. Stimatos, J.L. Multon (Eds.), Properties of Water in Foods in Relation to Quality and Stability, Martinus Nijhoff, Dordrech, 1985, pp. 661–679.
[28] L. Bell, T. Labuza, Determination of Moisture Sorption Isotherms. Moisture Sorption: Practical Aspects of Isotherm Measurement and Use, The American Association of Cereal Chemists, Inc., St. Paul, MN, USA, 2000, pp. 33–56.
[29] AOAC, Official Methods of Analysis 960.39, 15 th ed, Association of Official Analytical., Washington , DC, 1990.
[30] A. Durakova, N. Toshkov, A. Yovchev, Moisture Sorption characteristics of a floury mix with addition of Jerusalem Artichoke flour, J. Food Packag. Sci. Tech. Technol. 2 (2013) 215–219.
[31] A. Durakova, A. Bogoeva, V. Yanakieva, Moisture sorption characteristics and storage regime of defatted grape seeds flour – enological by-product, Asian J. Sci. Technol. 8 (11) (2017) 6794–6800.
[32] American Society of Agricultural Engineers, Moisture Relationships of Plant-Based Agricultural Products. ASAE Standard-2002, Standard Engineering Practices Data, ASAE Yearbook, 2002, pp. 538–550.
[33] C.J. Lomauro, A.S. Bakshi, T.P. Labuza, Evaluation of food moisture sorption isotherm equations part II: milk, coffee, tea, nuts, oilseeds, spices and starchy foods, LWT-Food Sci. Technol. 18 (2) (1985) 118–124.
[34] C.-C. Chen, R.V. Morey, Comparison of four EMC/ERH equations, Trans. ASAE 32 (3) (1989), 983-0990.
[35] L.B.H. Said, S. Bellagha, K. Allaf, Measurements of texture, sorption isotherms and drying/rehydration kinetics of dehydrofrozen-textured apple, J. Food Eng. 165 (2015) 22–33.
[36] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (2) (1938) 309–319.
[37] I. Ivanov, R. Vrancheva, A. Marchev, N. Petkova, I. Aneva, P. Denev, V. Georgiev, A. Pavlov, Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species, Int. J. Curr. Microbiol. App. Sci 3 (2) (2014) 296–306.
[38] T. Prokopov, A. Slavov, N. Petkova, V. Yanakieva, B. Bozadzhiev, D. Taneva, Study of onion processing waste powder for potential use in food sector, Acta Aliment. 47 (2) (2018) 181–188.
[39] BSS EN ISO 4833-1, Microbiology of Food Chain. Horizontal Method for the Enumeration of Microorganisms – Colony Count at 30°C by the Surface Plating Technique, Bulgarian State Standard, 2013.
[40] BSS EN ISO 21527-2, Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Determination of Yeasts and Fungi, Bulgarian State Standard, 2011.
[41] BSS EN ISO 16649-2, Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive *Escherichia coli* – Colony Count Technique at 44 Degrees C Using 5-Bromo-4-Chloro-3-Indolyl Beta-D-Glucuronide, Bulgarian State Standard (BSS), 2014.
[42] BSS EN ISO 6579-1, Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Detection of *Salmonella* Spp, Bulgarian State Standard, 2017.
[43] BSS EN ISO 6888-1, 2005/A1:2005, Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and Other Species), Bulgarian State Standard, 2005.
[44] L. Binzer, R. Brinsko, J. Cha, Z. Chen, S. Green, K. Grob, J. Hao, C. Hitz, L. Li, S. Swamy, Incorporating Grape Seed Antioxidants into a Functional Food Model, PhD Thesis, 2011.
[45] H. Lutterodt, M. Slavin, M. Whent, E. Turner, L.L. Yu, Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours, Food Chem. 128 (2) (2011) 391–399.
[46] S.-Y. Kim, S.-M. Jeong, W.-P. Park, K. Nam, D. Ahn, S.-C. Lee, Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts, Food Chem. 97 (3) (2006) 472–479.
[47] A. Al-Muhtaseb, W. McMinn, T. Magee, Moisture sorption isotherm characteristics of food products: a review, Food Bioprod. Process. 80 (2) (2002) 118–128.
[48] R.M. Lemus, Models of sorption isotherms for food: uses and limitations, Vitae 18 (3) (2011) 325–334.
[49] S. Brunauer, L.S. Deming, W.E. Deming, E. Teller, On a theory of the van der Waals adsorption of gases, J. Am. Chem. Soc. 62 (7) (1940) 1723–1732.