Einkorn (*Triticum monococcum L.*) and nectarine flour mixture – antioxidant activity, microbiological and sorption characteristics

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Abstract. The current paper presents the initial scientific research on a new enriched flour mixture with nectarine powder, comprising physicochemical parameters, antioxidant capacity, microbiological load, and sorption characteristics data. A mixture of 70% Einkorn flour and 30% fruit powder of Bulgarian origin as a functional food supplement was blended. The presence of bioactive components was proved by the *in vitro* analysis of antioxidant activity through DPPH, ABTS, FRAP, and CUPRAC methods. The microbiological indicators, the total count of mesophilic aerobic and facultative anaerobic microorganisms, yeasts and molds, *Escherichia coli*, *Salmonella* spp., coagulase-positive staphylococci and coliforms, were within the permissible norms and no presence of pathogenic microorganisms, were detected in the flour mixture. Adsorption and desorption processes were studied at 10, 25 and 40°C and a_w = 0.112-0.868 following static gravimetric methods. The obtained S-shaped equilibrium sorption isotherms confirm the constant trend that with increasing equilibrium moisture content, the temperature decreases in conditions of constant water activity. Based on the obtained sorption capacity data, a calculation of the monolayer moisture content was made using the linearization of the BET model. One of the modified three-parametric models of Oswin, Halsey, Henderson, and Chung-Pfost was selected as a suitable model for describing all sorption isotherms.

1 Introduction

In the last few years, food companies are refocusing their products on developing innovative, nutrient-rich products that meet consumer interests. Increased demand for various types of flour mixtures for bread, bakery and confectionery - fast to prepare and with a variety of combinations and flavors, provokes in-depth studies on foods with enriched functional composition [1-2].

Einkorn is a neglected type of wheat due to its difficult processing and low yield compared to other basic raw materials. It has a high level of nutritional values for its macro and micronutrients and a low gluten content when compared to other basic cereals, making it suitable and recommended for consumption in diets. In addition, einkorn contains a significant amount of fiber, antioxidants, protein and unsaturated fatty acids. It is rich in vitamins B and E, calcium, potassium, phosphorus, magnesium, sodium, selenium, iron and zinc. The combination of these qualities constitutes its therapeutic and prophylactic action on people suffering from cardiovascular disease and diabetes (due to the slow rise of blood sugar). Einkorn beneficially affects on reducing bad cholesterol, increasing nutrient absorption and promoting the work of the stomach intestinal tract. It also improves the immune response, making it a precaution against viral infections [3-5].

Nectarine, on the other hand, is a fruit with a peach-like shape, color, taste, and smell but with different chemical composition and a smooth surface. The health benefits of its consumption are numerous. One of them being: beneficial effects on the skin due to the high content of vitamins A and C; lowering blood pressure; preventing metabolic syndrome. Furthermore, nectarines are consumed to prevent cancer, hypertension, atherosclerosis, and are low in calories. The carbohydrates contained in nectarines slowly raise the blood sugar, making them suitable for diabetics. Their high fiber content favours the better functioning of the digestive system, and their high content of antioxidants

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reduces oxidative stress in the body. A significant advantage of nectarines over peaches is the lack of "fuzzy skin", which can provoke an allergic reaction. Therefore, nectarines are widely consumed, and in addition to fresh, can be found as compote, be added to desserts, fruit salads, cake decorations, cakes, or turned into jams, etc. [6-9].

The moisture content is one of the most monitored parameters, corresponding to and guaranteeing microbiological safety and maintaining good quality characteristics of powdered products. Conducting a study to determine the sorption capacity of food products provides information on the preparation and implementation of stages such as processing, drying, storage, and transport. Changing the equilibrium moisture content of the product according to the relative air humidity and temperature, can provoke unwanted physicochemical biological and microbiological reactions [10-12].

The possibility of mixing different types of flour and replacing a certain amount of basic flour with a functional nutrient provides a prerequisite for creating a new product with improved quality characteristics [13]. Based on an extensive literature review on research of enriched flour mixtures, to date there hasn't been found any mixture of einkorn flour and nectarine powder, intended for the production of sponge cakes. This gave us a reason to conduct the present study - the creation and determination of physicochemical, antioxidant, microbiological, and sorption characteristics of a mixture of einkorn flour and nectarine powder.

2 Materials and methods

2.1 Raw Materials

A flour mixture of 70% whole grain einkorn flour and 30% dried and powdered nectarines is composed. The study used einkorn flour produced by Eci-2002 LTD and purchased at a local food market in Plovdiv, Bulgaria. The physicochemical composition of einkorn flour for 100 g has already been determined by the manufacturer: energy value of 364 kcal; 2.97 g fat; 69.86 g carbohydrates; and 14.67 g protein. Nectarine fruits are purchased in local fresh stores in Plovdiv, Bulgaria. At the Institute of Food Preservation and Quality – Plovdiv, Bulgaria, fresh selected nectarine fruits are sliced until a thickness of 3.0 ± 0.5 mm and dried in a heat pump dryer for 8 h at 42°C and finely grounded with a Nutribullet blender. The physicochemical composition of nectarine powder per 100 g was determined to be: total lipids 0.25g; total carbohydrates 86.33 g; crude fibers 2.13 g; protein 1.98 g and ash content 4.87 g, calculated on a dry matter basis: 94.13% for nectarine flour.

2.2 Methods

2.2.1 Physicochemical parameters, antioxidant activity and microbiological load

Standard methods for the determination of chemical composition are: Ash content - ICC Standard No. 104/1 [14]; Proteins – AOAC 960.39 [15]; Total lipids and crude fiber - ISO 11085, 2015 [16]; Fibre – ISO 5489, 1981 [17]; Carbohydrates - AOAC Method 988.12 (44.1.30) [18]; Moisture content - AOAC 960.39 [15].

Antioxidant activity of the sample was investigated through the methods DPPH, ABTS, FRAP and CUPRAC detailed as described by Ivanov et al. [19-20] and Bogoeva et al. [20].

Standard methods for the determination of microbiological load are: Total count of mesophilic aerobic and facultative anaerobic microorganisms - BDS EN ISO 4833-1: 2013 [21]; Yeasts and molds - BDS EN ISO 21527-2: 2011 [22]; *Escherichia coli* - BDS EN ISO 16649-2: 2014 [23]; *Salmonella* spp. - BDS EN ISO 6579-1: 2017 [24]; Coagulase-positive staphylococci - BDS EN ISO 6888-1:2022 [25]; Coliforms – ISO 4832:2006 [26].

2.2.2 Sorption analysis and modelling of the data isotherms

Static-gravimetric method is used for determination the moisture sorption characteristics at 10, 25 and 40°C and relative air humidity within the range of 11% to 87% [11-13]. The investigation of the equilibrium moisture content and the experimental schema is presented in detail by Bogoeva [13]. Calculation of monolayer moisture content is performed through linearization of Brunauer-Emmett-Teller (BET) model Eq. 1 [27].

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

The obtained sorption data is described with four different three-parametrical modified model of Chung-Pfost (Eq. 2), Halsey (Eq. 3), Oswin (Eq. 4) and Henderson (Eq. 5), namely:

$$a_w = exp\left[\frac{-A}{t+B}exp(-CM)\right] \tag{2}$$

$$a_w = exp\left[\frac{-exp(A+Bt)}{M^C}\right] \tag{3}$$

$$M = (A+Bt)\left(\frac{a_w}{1-a_w}\right)^C \tag{4}$$

$$1 - a_w = exp[-A(t+B)M^C] \tag{5}$$

Non-linear function estimation analysis was conducted using the software "*STATISTICA*" (12.0) for the obtention of A, B and C coefficients.

These two criteria - mean relative error (*P, %*) Eq. (6), and standard deviation (*SEM*) Eq. (7) were chosen for estimation of the best model for the description of the obtained isotherms.

$$P = \frac{100}{N}\sum\left|\frac{M_i-\hat{M}_i}{M_i}\right| \tag{6}$$

$$SEM = \sqrt{\frac{\sum(M_i-\hat{M}_i)^2}{df}} \tag{7}$$

A detailed description of the sorption analysis is made by Bogoeva [13]. All samples were examined in triplicate runs.

3 Results and discussion

3.1 Chemical composition characterization of the flour mixture

The proximate composition of the flour mixture is: total lipids 1.91 ± 0.13%, protein 10.43 ± 0.27%, total carbohydrates 74.63 ± 0.55%, crude fibers 8.02 ± 0.31% and ash content 3.16 ± 0.24%. These results are calculated on a dry matter basis of 91.17 ± 0.10% for Einkorn/Nectarine flour mixture. The chemical analysis showed a decrease in the amounts of protein and lipids, as well as increased amounts of total carbohydrates and ash content compared to pure einkorn flour [3, 28-29]. These changes were attributed to the addition of nectarine powder, which is known from preliminary analyses to contain low amounts of protein and lipids and a large amount of total carbohydrates. Although nectarine peel, as a source of fibre, was not removed in the preparation of the nectarine flour, the amount of total fibre in the mixture was also reduced [3, 20].

3.2 Antioxidant activity

The percentage extract of a 1g sample is 32.36%.

Table 1. Antioxidant capacity of flour mixture of einkorn flour enriched with nectarine powder

Methods	mMTE/g extract	mMTE/g d.b.
DPPH	4.57 ± 0.15	1.48 ± 0.05
ABTS	1.63 ± 0.01	0.53 ± 0.01
FRAP	0.73 ± 0.02	0.23 ± 0.01
CUPRAC	2.32 ± 0.15	0.75 ± 0.05

The antioxidant capacity of the flour mixture was determined by four different methods. The flour mixture had the highest radical scavenging activity (DPPH = 4.57 ± 0.15 mMTE/g), followed by cupric reducing antioxidant capacity (CUPRAC = 2.32 ± 0.15 mMTE/g), antioxidant ability to scavenge ABTS (ABTS = 1.63 ± 0.01 mMTE/g) and ferric reducing antioxidant power (FRAP = 0.73±0.02 mMTE/g).

There is a known association between consumption of whole grain products and a reduced risk of cardiovascular disease and diabetes [30-31]. The antioxidant activity of einkorn is due to its carotenoids, polyphenols, and phytosterols as well as its high carotenoid and tocol levels [32]. As reported by Brandolini et al. [33], einkorn had a higher content of conjugated phenolic acids compared to bread wheat. The most abundant phenolic compound in einkorn is ferulic acid, followed by p-coumaric acid [33-34].

According to Stojanovic et al. [35], most of the polyphenols in nectarines are hydroxycinnamates. The same authors found three quercetin glycosides and one kaempferol glycoside in nectarine peels. In different studies neochlorogenic acid, catechin, and chlorogrnic acid represent 76 - 89% of total polyphenols in different varieties of nectarines [36]. Therefore, the addition of nectarine powder to einkorn flour results in an innovative product with higher antioxidant activity.

3.3 Microbiological load

The agricultural product einkorn flour is susceptible to microbial contamination during primary production and the milling process [4-6]. In a flour mixture of einkorn flour enriched with nectarine powder, the count of mesophilic aerobic and facultative anaerobic microorganisms showed levels of 6.7×10^4 CFU/g.

Table 2. Microbiological parameters of flour mixture of einkorn flour enriched with nectarine powder

Microbiological parameters	Results
Total number of mesophilic aerobic and facultative anaerobic microorganisms, CFU/g	6.7×10^4
Yeasts and molds, CFU/g	2.0×10^3
Coliforms, CFU/g	1.0×10^4
Coagulase-positive staphylococci, CFU/g	< 10
Escherichia coli, CFU/g	< 10
Salmonella spp.,/25 g	Not detected

Yeasts and molds are natural contaminants that together with aerobic bacilli fall into the flour of the field (soil, plants, etc.). Therefore, the presence of yeasts and molds at levels of 10^3 CFU/g is acceptable. The total coliform count was 1.0×10^4 CFU/g. The presence of coliforms indicates faecal and/or environmental contamination [37].

The flour is characterized by low water activity, and it is generally considered a microbiologically safe product [38]. Although pathogenic bacteria cannot grow in such conditions, pathogens that infect flour can survive for long periods of time [39]. In this study, the absence of *Salmonella* spp. was verified in 25 g and counts of Coagulase-positive staphylococci and *Escherichia coli* less than 10 CFU/g were observed. These results indicate that the flour mixture of einkorn flour enriched with nectarine powder is microbiologically safe.

3.4 Sorption characteristics of einkorn flour enriched with nectarines powder

As is well-known, the moisture-sensitive floury mixtures were monitored using the prediction and modelling of the sorption characteristics. The equilibrium moisture content demonstrates the relationship between moisture content and temperature in condition of constant relative air humidity and it was presented in Table 3 and Table 4 for the adsorption and desorption processes [10-13].

The tendency correlation - the augmentation of temperature degree affects the diminution of equilibrium moisture content for the same a_w, was confirmed [10, 12]. The graphical demonstration of the moisture sorption isotherms at 25°C was presented in Fig. 1.

The presented *S*-type sorption curve is from the second class of Brunauer's classification [40]. The isotherms were demonstrated the critical moisture and can being used to predict the potential quality loss in flour mixture [10-111; 13]. Four mathematical models by Oswin, Halsey, Henderson, and Chung-Pfost were proposed to fit the obtained moisture sorption curve (Table 5 for the adsorption process and Table 6 for the desorption process).

Table 3. Equilibrium moisture content (EMC) and standard deviation (SD) of Einkorn flour enriched with nectarine powder of Bulgarian origin for adsorption process

	10°C			25°C			40°C		
Sel	a_w	EMC	SD	a_w	EMC	SD	a_w	EMC	SD
LiCl	0.113	**7.23**	0.03	0.113	**6.45**	0.12	0.112	**5.01**	0.16
CH_3COOK	0.234	**9.36**	0.08	0.225	**7.99**	0.17	0.201	**7.08**	0.08
$MgCl_2$	0.335	**10.62**	0.10	0.328	**8.84**	0.14	0.316	**8.07**	0.14
K_2CO_3	0.431	**12.61**	0.17	0.432	**10.85**	0.05	0.432	**10.44**	0.09
$MgNO_3$	0.574	**16.08**	0.01	0.529	**12.32**	0.09	0.484	**11.97**	0.15
NaBr	0.622	**18.20**	0.04	0.576	**16.44**	0.07	0.532	**14.41**	0.09
NaCl	0.757	**28.20**	0.09	0.753	**23.50**	0.08	0.747	**22.66**	0.09
KCl	0.868	**36.16**	0.12	0.843	**34.29**	0.08	0.823	**33.60**	0.15

Table 4. Equilibrium moisture content (EMC) and standard deviation (SD) of Einkorn flour enriched with nectarine powder of Bulgarian origin for desorption process

	10°C			25°C			40°C		
Sel	a_w	EMC	SD	a_w	EMC	SD	a_w	EMC	SD
LiCl	0.113	**7.08**	0.07	0.113	**5.16**	0.17	0.112	**4.85**	0.16
CH_3COOK	0.234	**835**	0.05	0.225	**8.02**	0.07	0.201	**7.98**	0.09
$MgCl_2$	0.335	**10.09**	0.08	0.328	**8.44**	0.13	0.316	**8.42**	0.12
K_2CO_3	0.431	**13.66**	0.03	0.432	**11.63**	0.08	0.432	**10.20**	0.05
$MgNO_3$	0.574	**17.10**	0.07	0.529	**13.51**	0.05	0.484	**12.56**	0.02
NaBr	0.622	**18.76**	0.15	0.576	**14.63**	0.09	0.532	**12.64**	0.12
NaCl	0.757	**23.44**	0.12	0.753	**22.85**	0.04	0.747	**22.25**	0.05
KCl	0.868	**44.59**	0.14	0.843	**36.30**	0.03	0.823	**32.96**	0.08

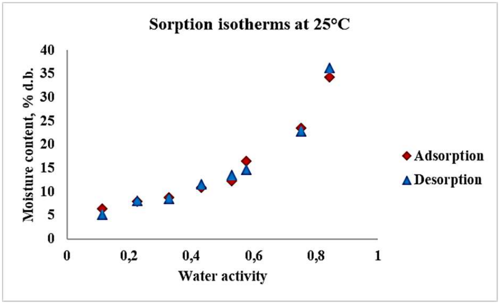

Fig. 1. Sorption isotherms at 25°C

Table 5. Coefficient of three-parametrical models (A, B and C), mean relative error (P, %) and standard deviation (SEM) of Einkorn flour enriched with nectarine powder of Bulgarian origin for adsorption process

Model	Oswin	Halsey	Henderson	Chung-Pfost
A	14.311	**3.611**	0.0002	1.942
B	-0.017	**-0.009**	2.639	-1.686
C	0.524	**1.460**	1.918	0.087
P	9.61	**6.06**	20.09	87.21
SEM	1.54	**1.93**	5.57	18.47

Table 6. Coefficient of three-parametrical models (A, B and C), mean relative error (P, %) and standard deviation (SEM) of Einkorn flour enriched with nectarine powder of Bulgarian origin for desorption process

Model	Oswin	Halsey	Henderson	Chung-Pfost
A	14.739	**3.493**	0.0001	464.656
B	-0.051	**-0.007**	2.661	0.109
C	0.577	**1.427**	1.929	117.352
P	10.48	**6.21**	24.79	13.49
SEM	1.72	**1.33**	5.91	4.41

As shown in Table 5 and Table 6, we can recommend the modified three-parametrical model of Halsey for the best satisfactory described the obtained adsorption and desorption S-shaped curves.

The BET model was used to determine the monolayer moisture content. The linearization of the obtained Brunauer-Emmett-Teller isotherms was demonstrated in Figure 2 for the adsorption process and on Fig. 3 for the desorption process [27]. All results were shown for aw ≤ 0.5.

The monolayer moisture content was calculated to obtain the general point of moisture stability, guaranteeing no food deterioration [10 - 13]. The values for the adsorption process are 7.32% at 10°C, 6.17% at 25°C and 6.23% at 40°C. For the desorption process are: 7.89% at 10°C, 6.93% at 25°C and 6.12% at 40°C.

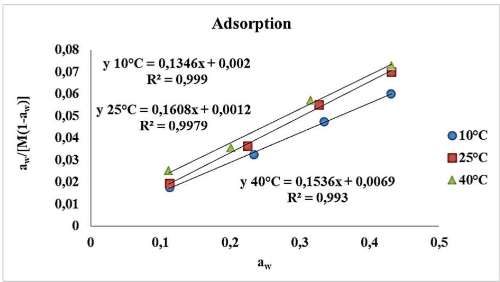

Fig. 2. BET model linearization for adsorption

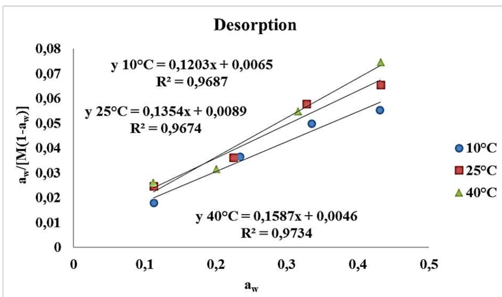

Fig. 3. BET model linearization for desorption

4 Conclusion

The new flour mixture intended for sponge cake production of Einkorn flour and nectarine powder was created. The selected analyses were investigated:

1. Physicochemical parameters: total lipids 1.91%, protein 10.43%, total carbohydrates 74.63%, crude fibers 8.02% and ash content 3.16%.

2. Antioxidant activity was proved using the DPPH, ABTS, FRAP and CUPRAC, methods.

3. The investigated parameters: the total count of mesophilic aerobic and facultative anaerobic microorganisms, yeasts and moulds, *Escherichia coli*, *Salmonella* spp., coagulase-positive staphylococci and coliforms, indicated the microbiological safety.

4. Equilibrium moisture content for the adsorption process is within the range of 5.01% d. b. and 36.16% d.b. and for the desorption process between 44.59% d.b. and 4.85% d.b.

5. All obtained sorption isotherms have S-type curve characteristics for the most food powdered products, according to Brunauer's classification.

6. For fitting the selected modified three-parametric models of Oswin, Halsey, Henderson, and Chung-Pfost, we recommend the Halsey model as the most suitable model describing the obtained sorption isotherms.

7. After linearizing the BET model, the monolayer moisture content was calculated. The adsorption process has a range of 6.17% d. b. to 7.23% d. b., and the desorption process has a range of 6.12% d.b. to 7.89% d.b.

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