

Antioxidant activity and sorption characteristics of ready-made mixture with lucuma powder

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Abstract

Bogoeva, A., Durakova, A., Vrancheva, R. & Slavov, A. (2020). Antioxidant activity and sorption characteristics of ready-made mixture with lucuma powder. *Bulg. J. Agric. Sci.*, 26 (4), 906–912

The current scientific research is focused on a new ready-made mixture for a cream-like product with a powdered subtropical fruit Lucuma. We performed an analysis on the antioxidant activity via four analytical methods differing in their mechanism and reaction conditions, namely DPPH, ABTS, FRAP and CUPRAC. The obtained data were expressed as mM TE/g powder (3.00 ± 0.01 ; 7.02 ± 0.24 ; 4.08 ± 0.23 ; 15.40 ± 0.61 , respectively). Equilibrium moisture content for adsorption and desorption process was determined using the static gravimetric method of saturated salt solution at three different temperatures – 10°C, 25°C and 40°C and relative humidity from 0.11 to 0.90. According to the obtained data the sorption capacity decreases when the temperature increases under the conditions of constant water activity. In order to describe the obtained sorption isotherms we used four different mathematical models – modified Chung-Pfost, modified Oswin, modified Halsey and modified Henderson. The results showed that the modified Halsey model could be recommended for a satisfactory description of the mixture sorption isotherms. The monolayer moisture content was calculated using Brunauer-Emmett-Teller (BET) equation whose results for adsorption was within the range of 2.52% to 4.41% and for desorption – from 2.15% to 3.56%

Keywords: lucuma; sorption isotherms; ready-made; cream-like; mixture; antioxidant activity; adsorption; desorption

Introduction

Enhanced consumer interest in enriching the nutrition menu sets a prerequisite for expanding the food assortment by new products not typical of the geographical region. The selection is based on their composition and the degree of enrichment with important micro- and macro-nutrients (Verbeke, 2006). The widespread demand for biologically active sources is due to their good impact on the immune system and the positive effects of their consumption such as the prevention of a number of diseases (Hennig et al., 2007; Guendez et al., 2005). For an optimal maintenance of vital functions the cells in every living organism must produce oxygen. One of the main preventives is the daily procure-

ment of antioxidants from natural sources – fruits, vegetables, plants and others (Bagchi et al., 2000; Eastwood, 1999, Pinto et al., 2009). Nowadays, there has been an increasing trend in both production and application of ready-made powder mixtures composed entirely of dry ingredients. Their use does not require mixing, homogenization and storage of the components separately which facilitates their application (Dimov, 2014).

Lucuma powder is a kind of fruit flour also known as “Inca Gold” (Caballero & Aguilar, 2017). Exotic fruit is often used as a sugar substituent due to its characteristic caramel odor and taste (Aguilar, 2015; Taiti et al., 2017). For the Bulgarian market it is a new product, part of the assortment “Super foods”.

For a good and adequate selection of the flour storage regime, the environmental parameters initially are taken into account, i.e. temperature and relative air humidity and their effect on the moisture of the product. These factors are the basis of the sorption characteristics studies which provide the necessary information on the conditions for processing, storing, packaging and transporting different food products. The main sorption characteristics for food products are the equilibrium moisture content. The relation between the equilibrium moisture content (M) and the water activity a_w is represented by the experimentally constructed sorption isotherm for a specific temperature (t) – $M = f(a_w, t)$ (Dupas-Langlet et al., 2016; Kaya & Kahyaoglu, 2005; Muzaffar & Kumar, 2016). The value of the moisture corresponding to the monolayer moisture content is an essential sorption characteristic. It affects the stability of the product (Timmermann, 2003; Troller, 2012).

Materials and Methods

Commercial Lucuma powder, produced in Peru, purchased in Bulgaria by “Internet café-BG” Ltd, packed by “Zoya bg Organic Shop” was used in this study. In order to create the ready-made mixture we used dried milk 1.5% fat purchased from “Bioset” Ltd and the powdered Guma Arabica designed for food industry was delivered by “Panteley Toshev” Ltd.

The approximate average physicochemical composition of the investigated products of the new mixture was determined according to the Association of Official Analytical Chemist (AOAC), 2005 respectively AOAC (2005) Determination of Moisture, Ash, Protein and Fat (Official Method of Analysis of the Association of Analytical Chemists. 18th Edition, AOAC, Washington DC).

Carbohydrates, % is calculated as the difference between 100% total mass and the percentage of fat, protein, ash, and moisture content (Ferris et al., 1995).

The total dietary fibers, insoluble dietary fibers and soluble dietary fibers were determined using K-TDFR-100A (Megazyme, Ireland) according to AOAC method 991.43 “Total, soluble and insoluble dietary fibers in foods” (First action 1991) and AACC method 32-07.01 “Determination of soluble, insoluble and total dietary fibers in foods and food products” (Final approval 10-16-91).

Antioxidant activity

Sample extraction for analysis of antioxidant activity

The analyzed samples were subjected to triple extraction with 10 mL 70% ethanol in a water bath at a temperature of 70°C under a reflux condenser. The combined extracts were filtered through filter paper and used for further analysis. The

antioxidant activity was determined based on the following methods:

DPPH assay

Each analyzed extract (0.15 mL) was mixed with 2.85 mL freshly prepared 0.1 mM solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in methanol. The reaction was at 37°C in darkness and the absorbance at 517 nm was recorded after 15 min against methanol.

ABTS assay

ABTS radical was generated by mixing aliquot parts of 7.0 mM 2,2'-azinobis (3)-ethylbenzthiazoline-6-sulfonic acid (ABTS) in distilled H₂O and 2.45 mM potassium persulfate in distilled H₂O. The reaction was performed for 16 h at ambient temperature in darkness and the generated ABTS radical remains stable for several days. Before analyses, 2.0 mL of generated ABTS + solution was diluted with methanol in proportions 1:30 (v/v), so the obtained final absorbance of the working solution was about 1.0±1.1 at 734 nm. For the assay, 2.85 mL of this ABTS + solution was mixed with 0.15 mL of obtained extracts. After 15 min at 37°C in darkness, the absorption was measured at 734 nm against methanol.

Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was freshly prepared before each analysis by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ, Fluka) in 40 mM HCl (Merck) and 1 part 20 mM FeCl₃·6H₂O (Merck) in distilled H₂O. The reaction was started by mixing 3.0 mL FRAP reagent with 0.1 mL of the investigated extract. Blank sample, prepared with 70 % ethanol instead of extract was developed as well. The reaction time was 10 min at 37°C in darkness and the absorption at 593 nm of sample against blank was recorded.

Cupric reducing antioxidant capacity (CUPRAC) assay

Reaction was started by mixing 1.0 mL 10 mM CuCl₂·2H₂O (Sigma) in distilled H₂O, 1.0 mL 7.5 mM Neocuproine (Sigma) in methanol, 1.0 mL 0.1 M ammonium acetate buffer (pH 7.0), 0.1 mL of investigated extract and 1.0 mL distilled H₂O. Blank sample, with 70 % ethanol instead of extract was developed as well. The reaction was carried out for 20 min at 50°C in darkness and the sample absorption at 450 nm was recorded against the blank.

The antioxidant activity defined by all of the tested methods was expressed as mM Trolox equivalents (TE) per g dry weight (DW) and g extract by using calibration curve, built within the range of 0.05–0.5 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) dissolved in methanol.

Sorption characteristics

The equilibrium moisture content (EMC) of the ready-made mixture was investigated at 10°C, 25°C and 40°C and $a_w = 0.11 \div 0.90$ using the static gravimetric method (Wolf et al., 1985). The powder was dried in a desiccator with P_2O_5 at a room temperature for 20 days prior to the beginning of the experiment for the adsorption process. For determination of the desorption process the samples were hydrated in a glass jar over distilled water at a room temperature. Samples of $1 \div 0.02$ g were weighed in weighing bottles. Eight saturated salt solutions (LiCl, CH_3COOK , $MgCl_2$, K_2CO_3 , $MgNO_3$, NaBr, NaCl, KCl) maintaining constant water activity environments were used in the weighing bottles which were put in hygrometers (Bell & Labuza, 2000). All of the used salts were of reagent grade. At high water activities ($a_w > 0.70$) crystalline thymol was placed in the hygrometers to prevent microbial spoilage of the flour. The hygrometers were kept in thermostats at 10°C, 25°C and 40°C $\div 0.2^\circ C$. The samples were weighed (balance sensitivity $\div 0.0001$ g) every three days. Equilibrium was ascertained when three consecutive weight measurements showed a difference less than 0.001 g. The moisture content (%) was calculated according to AOAC 960.39.

For verification of the description of sorption isotherms we used the following models:

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.; a_w is the water activity, decimal; A , B and C are coefficients; t is the temperature, °C.

A non-linear 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 & 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: 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).

The monolayer moisture content (MMC) is calculated using the Brunauer-Emmett-Teller (BET) equation and the experimental data for water activities up to 0.45 for each temperature (Brunauer et al., 1938; Bell & Labuza, 2000; Durakova et al., 2013):

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

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

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

However, we were not able to find any information concerning physicochemical composition, antioxidant activity and sorption characteristics of the ready-made mixture for the cream-like product with powdered subtropical fruit *Lucuma*.

Results and Discussion

Initial analyses of a new ready-made mixture for a cream-like product with powdered subtropical fruit *Lucuma* are begun with determining of the physicochemical parameters – proteins, carbohydrates, moisture, fat and ash content. The approximate average physicochemical composition of the used ingredients (according to information on packages) and the final new product are presented in Table 1.

The aim was to create an instant product which could be prepared with cold water. Most instant creams on the market consist of sugar, modified corn starch, different flavours, colorants and gelling agents as carboxymethyl cellulose E466. We used *Lucuma* powder as a source of color, flavour, antioxidant activity and sugar substituent (Fuentelba et al., 2016; Dini, 2011; Rojo, 2010). Moreover, this is a way to avoid numerous ingredients.

The dietary fibres consumption is related to favorable and beneficial effects upon human health in daily nutrition.

Table 1. The approximate average physicochemical composition of the products

Sample	Proteins, %	Carbohydrates, %	Moisture, %	Fat, %	Ash, %
Ready-made mixture for cream-like product	24.1	66.8	6.4	1.5	1.2
Lucuma powder	4	82.2	10.7	1.1	2
Dried milk	32	55	6.1	1.5	5.4
Gum Arabic SD, E-414	2.1	90	6.9	0.9	0.1

The fibres slow intestinal transit, delays gastric emptying, and reduce glucose and cholesterol absorption by the intestine. The soluble fraction was found to be an important factor for normalizing serum lipid levels and decreasing the postprandial glucose response. The lucuma fruits were found to be a rich source of dietary fibres: 32.47–35.72% for the different varieties (Glorio et al., 2008). Addition of lucuma lead to functionalization of the product and it was found to contain significant amounts of dietary fibres: 20.36±1.03%, 3.35±0.86% from which are soluble, and could be considered as source of fibres having in mind that according to the European legislation such claim is permitted if the food systems have more than 3 g per 100 g (Nath et al., 2018). The agro industrial wastes from *Pouteria* sp. processing were also found to be a good source of antioxidants (Guerrero-Castillo et al., 2019).

According to our analyses the new ready-made mixture contains total dietary fibers (TDF): 20.36±1.03, insoluble dietary fibers (IDF): 17.01±1.01 and soluble dietary fibers (SDF): 3.35±0.86.

Antioxidant activity

An extract of 23 ml was obtained after an extraction of 1 g of the ready-made mixture following the described procedure. The antioxidant activity of the obtained 70 % ethanol extract was determined by four methods based on different mechanisms and reaction conditions – DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azobis (3)-ethylbenzothiazoline-6), FRAP (ferric reducing antioxidant power) and CUPRAC (cupric reducing antioxidant capacity). The methods based on a single electron transfer (SET) and/or a hydrogen atom transfer (HAT method) are DPPH and ABTS and the methods based on single electron transfer (SET method) are FRAP and CUPRAC. The obtained results (mean ± standard deviation) are presented in Figure 1 expressed as mM TE/g ready-made mixture.

Our results confirmed that the analyzed liquid extract of a new ready-made mixture for cream-like product with powdered subtropical fruit Lucuma possessed antioxidant activity defined by all of the tested methods which is evident from Figure 1. Therefore, small quantities of incorporated Lucuma powder could have beneficial health effects. There

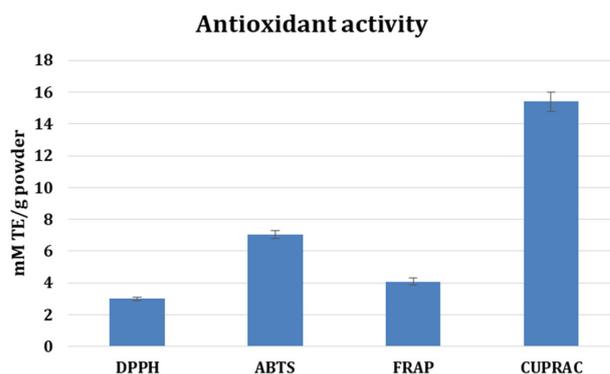


Fig. 1. Antioxidant activity of 70 % ethanol extract of the ready-made mixture expressed as mM TE/g powder

were no available data in the scientific literature concerning the antioxidant activity of ready-made mixture for cream-like product with Lucuma powder. According to Guerrero-Castillo et al. (2019) the methanol extract of *Pouteria Lucuma* seeds possessed antioxidant activity determined by DPPH, ABTS and FRAP methods (58.14 ± 0.05 µg/ml, 66.97 ± 0.00 µg/ml, 272.50 ± 0.00 µmol Trolox/g dry weight, respectively). The presence of antioxidant activity in *Sapoteseae* family, specifically fresh fruits of *Pouteria campechiana*, *Pouteria sapota* and *Pouteria viridis* is confirmed in Jun Ma's thesis, 2004. High antioxidant capacity of Lucuma extract was also reported by Yahia and Guttierrez-Orozco, 2011.

Moisture sorption analysis of ready-made mixture

The obtained mean values of EMC based on triplicate measurements for the respective water activity and temperature are presented in Figure 2 for adsorption and in Figure 3 for desorption.

The results of the EMC increase with an increase in the temperature at a constant a_w . We found similar results which were reported in the scientific research on the sorption isotherms (Al-Muhtaseb et al., 2002; Muzaffar & Kumar, 2016; Decagon, 2011).

Figure 2 and Figure 3 give the experimental data obtained after adsorption and desorption at 10°C, 25°C, 40°C.

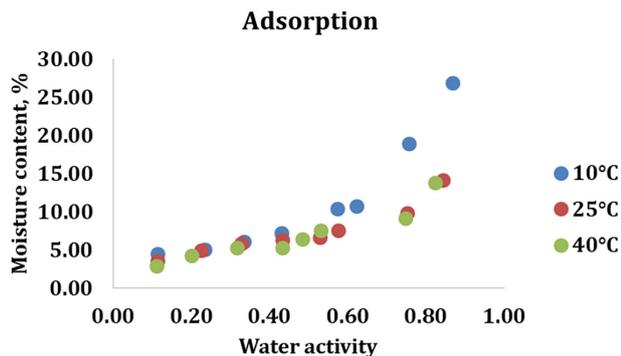


Fig. 2 Sorption isotherms of the mixture for the adsorption process at different water activities (a_w) and temperatures t ($^{\circ}\text{C}$).

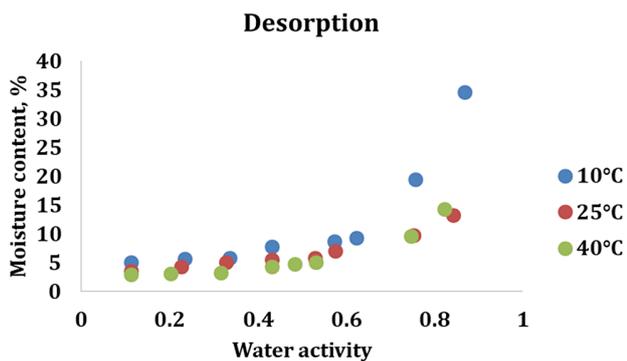


Fig. 3 Sorption isotherms of mixture for desorption process at different water activities (a_w) and temperatures t ($^{\circ}\text{C}$).

The sorption isotherms have an *S*-shape profile according to the classification of Brunauer et al. (1940).

The hysteresis effect is statistically significant at a level of significance $\alpha = 0.05$ 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 2 for adsorption and Table 3 for desorption.

According to the results the lowest values of P and SEM were obtained with the Halsey model.

The model (8) is linearly transformed for the calculation of the BET monolayer moisture content (MMC):

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

Based on the coefficients of the linear equation the MMC for the respective temperature is calculated and the results are presented in Table 4.

Table 4. BET monolayer moisture content MMC (% d.b.) of the mixture at several temperatures

t ($^{\circ}\text{C}$)	Adsorption	Desorption
10	4.41	3.56
25	2.52	2.15
40	3.32	2.43

Conclusions

The approximate composition of the new ready-made mixture was determined. The antioxidant activity was proven via DPPH, ABTS, FRAP and CUPRAC expressed as mM TE/g powder (3.00 ± 0.01 ; 7.02 ± 0.24 ; 4.08 ± 0.23 ; 15.40 ± 0.61 , respectively). The sorption capacity of the investigated product decreases with an increase in temperature at constant wa-

Table 2. Model coefficients (A , B , C), mean relative error (P , %) and standard error of moisture (SEM) for adsorption

Model	A	B	C	P	SEM
Oswin	10.78775	-0.12975	0.50043	18.78	2.09
Halsey	3.419866	-0.01410	1.72757	10.09	1.68
Henderson	0.000347	1.66885	2.15421	18.42	2.87
Chung-Pfost	338.9116	0.24669	50.5481	15.68	2.37

Table 3. Model coefficients (A , B , C), mean relative error (P , %) and standard error of moisture (SEM) for desorption

Model	A	B	C	P	SEM
Oswin	11.94475	-0.19134	0.59908	26.99	2.59
Halsey	3.374496	-0.02971	1.57616	10.02	1.39
Henderson	0.001520	9.9736	1.33199	21.73	1.97
Chung-Pfost	149.6136	0.21686	21.1343	24.57	2.60

ter. The modified Halsey model is suitable for describing the relation between the equilibrium moisture content, the water activity and the temperature of the new ready-made mixture. According to the sorption isotherms obtained for 10°C, 25°C and 40°C MMC is calculated with the BET equation (for the adsorption process – 2.52% to 4.41% and for the desorption – from 2.15% to 3.56%).

Acknowledgments

This study was part of the National Scientific Programme “Young Researchers and Postdocs” at the Technical faculty, University of Food Technologies – Plovdiv, Bulgaria with the funding of Fund Scientific Research “FS N07/18-H”, project director: Assoc. Prof. PhD Albena Georgieva Durakova, University of Food Technologies – Plovdiv, Bulgaria.

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Received: July, 17, 2019; Accepted: July, 24, 2019; Published: August, 31, 2020