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PROCESSING METHODS FOR THE JUICE OF BITTER MELON FRUITS
METODE PRERADE SOKA OD GORKE DINJE

Simona PERŢA-CRIŞAN, Raul IANCHICI
"Aurel Vlaicu" University, Faculty of Food Engineering, Tourism and Environment Protection,
310330 Arad, Elena Drăgoi 3, Romania
e-mail: simo_crisan@yahoo.com

ABSTRACT

Momordica charantia L. belongs to Cucurbitaceae family, being commonly named bitter melon. It represents an important source of active principles with antidiabetic action. Juice of the fruits is known to exhibit hypoglycemic properties, being recommended to reduce blood sugar levels in patients suffering of diabetes mellitus. Our researches focused on studying two protein fractions from the juice of bitter melon fruits, compounds with proved hypoglycemic activity: polypeptide-p and peptide MC6. In order to preserve these biological active compounds we have proceeded to drying fresh juice from fruits through three different methods: pulverization drying, vacuum drying and lyophilization. For further qualitative analysis we have reconstructed the juice by rehydration. Separation of all protein fractions and identification of the two compounds from juices were realized by SDS-PAGE electrophoresis. Results have suggested that studied compounds have not been damaged by thermal treatments, which constitute good processing and preserving methods.

Key words: *bitter melon, polypeptide-p, peptide MC6, pulverization drying, vacuum drying, lyophilization.*

REZIME

Momordica charantia L. pripada porodici Cucurbitaceae, uobičajeno se zove gorka dinja. Predstavlja značajan izvor aktivnih supstanci sa antidijabetskim dejstvom. Za voćni sok poznato je da ispoljava hipoglikemička svojstva, što je preporučljivo za smanjenje nivoa šećera u krvi kod obolelih od dijabetesa. Istraživanja su usmerena na proučavanje dve proteinske frakcije iz soka gorke dinje, jedinjenja sa dokazanim hipoglikemičkim dejstvom: polipeptid-p i peptid MC6. U cilju očuvanja ovih biološki aktivnih jedinjenja obavljeno je sušenje svežeg soka od voća sa tri različita metoda: pulzaciono sušenje, vakuum sušenje i liofilizacija. Za dalje kvalitativne analize rekonstruisan je sok rehidracijom. Razdvajanje svih proteinskih frakcija i identifikacija dva jedinjenja iz sokova obavljena je SDS-PAGE elektroforezom. Rezultati su pokazali da ispitivana jedinjenja nisu oštećena termičkim tretmanom, koji predstavljaju dobar način za preradu i konzerviranje.

Ključne reči*: gorka dinja, polipeptid-p, peptid MC6, pulzaciono sušenje, vakuum sušenje, liofilizacija.*

INTRODUCTION

Native to Asia, bitter melon (*Momordica charantia* L.) is cultivated especially throughout the tropics, but can accommodate itself to other countries' warm climate, like western part of Romania (*Crisan, Simona et al., 2009a*). All parts of plant have been used since ancient times in traditional medicine, being considered in numerous countries from Asia one of the most common medicinal plants, extremely valuable because of its hypocholesteric (*Chatuverdi, 2005; Senanayake et al., 2004*), antiviral (*Jiratchariyakul et al., 2001*), antibacterial (*Khan et al., 1998*), anticancerous (*Nagasawa et al., 2002*) effects and most of all because of its hypoglycemic-antidiabetic strong action (*Reyes et al., 2006; Shetty et al., 2005; Zheng et al., 2005*). Various parts of plant, especially the fruit, have been widely used for preparation of hypoglycemic pharmaceutical compositions (*Ianculov et al., 2010*). The blood sugar lowering action of the fresh juice has been clearly established in both experimental and clinical studies, also acting as regenerating of damaged pancreatic cells with significant increase of insulin secretory activity (*Ahmed et al., 2001; Cummings et al., 2004*). Oral administration of 50-100 ml of the juice has shown good results in clinical trials (*Abascal, Kathy and Yarnell, 2008*). Among chemical compounds with demonstrated hypoglycemic activity can be noticed: charantin (steroidal glycoside), vicine (alkaloid present in seeds), lectins and polypeptide-p/p-insulin/v-insulin (insulinomimetic proteins), peptide MC6. The action mechanism of these compounds is not clarified, not existing till present any conclusions if any of these active compounds acts alone or their action is synergic. Hypoglycemic effect is more pronounced in fruit, where these compounds are prevalent. Polypeptide-p has been isolated from fruit, seeds, and tissue of bitter melon. It is a very effective hypoglycemic agent when administered subcutaneously to gerbils and humans (*Khanna et al., 1981*). Peptide MC6 comprises three peptides: MC6.1 and its derivates MC6.2 and MC6.3, which are also peptides, but shorter in length. This active fraction exhibits a hypoglycemic activity and may be administrated orally to treat a variety of hyperglycemic disorders (*Bishwajit et al., 2005*). Protein analysis by SDS-PAGE electrophoresis indicates for polypeptide-p a molecular weight of approximately 11,3 kDa, while peptide MC6 is characterized by a molecular weight of about 9,9 kDa and a movement as a single band on SDS-PAGE electophoresis (*Crisan, Simona et al., 2009b*).

Nomenclature:

MW (Da)	– molecular weight
p (Pa)	– pressure
Rf	– retention factor
t (˚C)	– temperature
v (m/s)	– velocity
φ (%)	– air relative humidity

Subscripts

ca	– compressed air
dt	– dry thermometer
wa	– warm air
wt	– wet thermometer

MATERIAL AND METHOD

Bitter melon fresh unripe fruits obtained from our own field in the western part of Romania, Arad County, were chopped into small pieces and the seeds removed. Fruits were then homogenized using a knife mill (GRINDOMIX GM 200, Retsch, Ger-

many). Fresh juice was first filtrated making use of a polypropylene mesh woven filter (Spectrum Laboratories, USA) and then centrifuged at 5000 rpm employing Hettich EBA 20 centrifuge (Germany). Supernatant wad dried through some different methods: pulverization, under reduced pressure and lyophilization, retaining one sample of fresh juice as standard. Within pulverization or atomization drying method, juice was fine pulverized using a spray gun (M22 G HTi, Kremlin Rexson and Sames, Germany) coupled to an air compressor (BT-AC 250/50, Einhell, Germany) in warm parallel air flow of a tray dryer (UOP 8 Tray Dryer, Armfield, UK), so that sprayed particle temperature did not exceed 30˚C. Working parameters were: v_{wa} 0,97 m/s, t_{dt} 60˚C, t_{wt} 29˚C, φ 18%, p_{ca} 6 bar. For vacuum drying, juice was dried by reducing working pressure, which resulted in drying at a lower temperature than is required at full pressure and unwanted active principles wastes eliminated. This procedure implied a SpeedVac concentrator (SPD 111V-230, Thermo Electron Corporation, USA), coupled to a vacuum pump (PC 2002 Vario with CVC 2000 controller, Vaccubrand, Germany). Juice was first introduced in 1,5 ml microcentrifuge tubes hold in RD36 Rotor of concentrator and then set working parameters: t 35±1˚C and Manual Run function mode. Lyophilization drying involved juice prior freezing at –55˚C and ice sublimation at a vacuum of 0,10 mbar, using for this procedure a freeze dryer (Christ Alpha 1-4 LD Plus, SciQuip, UK). For further proteins analysis, dried juices were reconstructed by rehydration, so that final working juice samples were ten times concentrated relative to initial juice. In the same time, standard fresh juice sample was also ten times concentrated using SpeedVac concentrator. In order to separate protein fractions and identify polypeptide-p and peptide MC6 from various juice samples, it was applied SDS-PAGE electrophoresis using for this purpose an electrophoresis special device (Mini Vertical Gel System EC120, Thermo Electron Corporation, USA coupled to Consort EV265 Power supply) and specific reagents (Amresco Inc., USA): Fluorescent SPRINT NEXT GEL 10%, APS/TEMED polymerization tablets, Sample loading buffer 4X, NEXT GEL running buffer 20X, Coomassie Briliant Blue R250, K880 low range protein MW marker (6 bands ranging from 3.5 to 31.0 kDa), K494 wide range protein MW marker (8 bands ranging from 14.0 to 212.0 kDa). For preparing gel plates were followed steps described in product's technical support *(***, 2008*). Prior to electrophoretic analysis, samples were prepared as described in Table 1, final solutions being electrophoretically separated and analyzed.

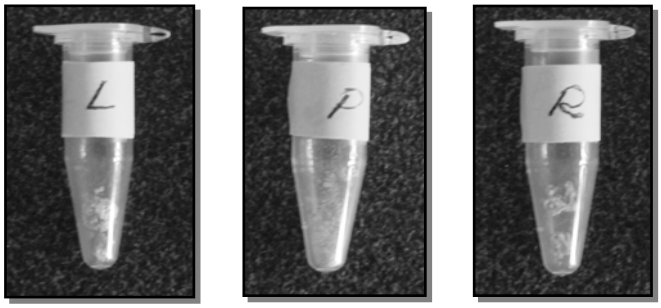
Table 1. Samples preparation for electrophoretic analysis

	Juice sample (Standard)	1	2	3	k880 MW marker	k494 MW marker
Drying method		Lyophilization	Pulverization	Vacuum drying		
Solution quantity	60 µl	60 µl	60 µl	60 µl	30 µl	30 µl
Sample loading buffer	20 µl	20 µl	20 µl	20 µl	10 µl	10 µl
Warm in water bath (60-70˚C)	2-3 min.	2-3 min.	2-3 min.	2-3 min.	-	-
Boil in water bath	-	-	-	-	2-3 min	2-3 min
Cool	Several minutes					
Transfer on plates	20 µl	20 µl	20 µl	20 µl	10 µl	10 µl
Electrophoresis parameters	200 V, 40 minutes (Fluorescent SPRINT NEXT GEL 10%)					

After electrophoresis performing, a simple one step Coomassie Blue staining method for protein detection was applied (*Chen et al., 1993*). Stained gel was water washed and photographed for making the interpretation. In order to appreciate protein fractions molecular weights was drawn Rf vs. logMW calibration curve for MW markers, while for identifying the compounds of interest polypeptide-p and peptide MC6 was first determined their zone in the lane and then created its density profile by scanning and analyzing with a specialized software UN-SCAN-IT gel - Gel Analysis Software.

RESULTS AND DISCUSSION

Processing methods applied to juice of bitter melon fruits, meaning lyophilization drying, pulverization drying and vacuum drying, led to a product in powder form (Figure 1). All possessed bitter taste and specific smell. Pulverized juice presented a finer and more homogeneous structure than the other two. Dried juice was characterized by high solubility, maximum moisture of 5-7% and extreme hygroscopicity. That is the reason why it is really necessary to pack the product, narrow and rapidly, in special plastic inside metallic bags with tight vacuum-assisted closure performed by hot welding. Stored at appropriate temperature and humidity, the product retains its properties over a long period.



a *b* *c*

Fig. 1. Dried juice
a – Lyophilized; b – Pulverized; c - Vacuum dried

Protein fractions contained by juice samples, separated in accordance with their molecular weights, were visually identified and shown in Figure 2 and Figure 4. Concordantly to gel photographs, it was realised the diagrammatic disposal of bands corresponding to protein fractions.

According to electrophoregram of protein fractions from standard juice sample (Figure 2), fractions F8 (MW 33 kDa), F6 (52 kDa), F5 (70 kDa) and F7 (45 kDa) were predominant, but the zone of interest for our research (polypeptide-p and peptide MC6) proved to be F13-F16 grouped protein fractions (Figure 4).

After gel scanning and analyzing, there were identified the two protein fractions of interest as F15 and F16, which corresponded to compounds with molecular weights of about 11300 Da and 9900 Da (*Crisan, Simona et al., 2009b*).

Comparing electrophoregram of protein fractions from standard juice sample (Figure 2) with electrophoregram of protein fractions from rehydrated dried juices samples (Figure 3), one can observe the presence in all lanes of same protein fractions as in standard juice sample (F1, F2, ..., F16).

Although some protein fractions were less visible in the lanes of rehydrated dried juices, zone F13-F16 of the two studied compounds with hypoglycemic activity was the same, including same protein fractions as standard juice.

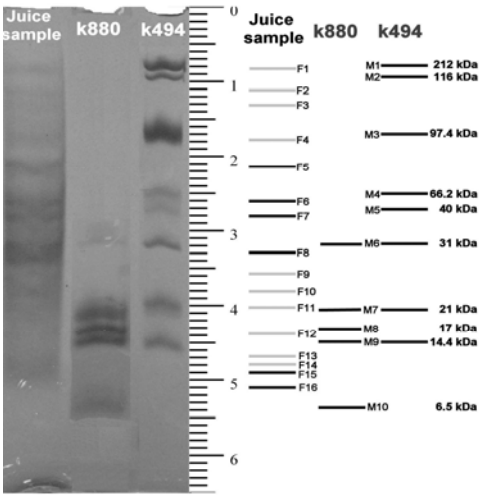


Fig. 2. Electrophoregram of protein fractions from standard juice and MW markers
(F5-F8 – predominant protein fractions, F1-F4, F9-F16 – less predominant protein fractions, M6-M10 – low range protein fractions (k880 MW marker), M1-M7 and M9 – wide range protein fractions (k494 MW marker))

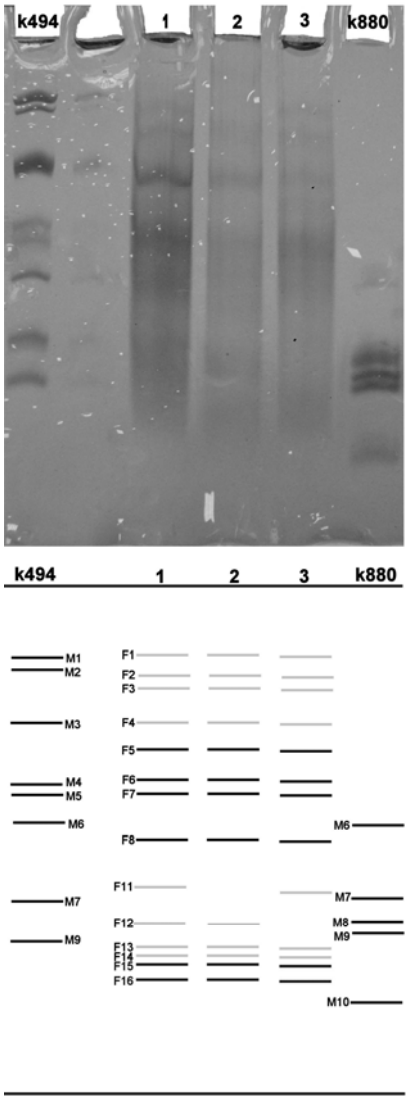


Fig. 3. Electrophoregram of protein fractions from rehydrated dried juices and MW markers
1 – Lyophilized juice lane; 2 – Pulverized juice lane; 3 – Vacuum dried juice lane

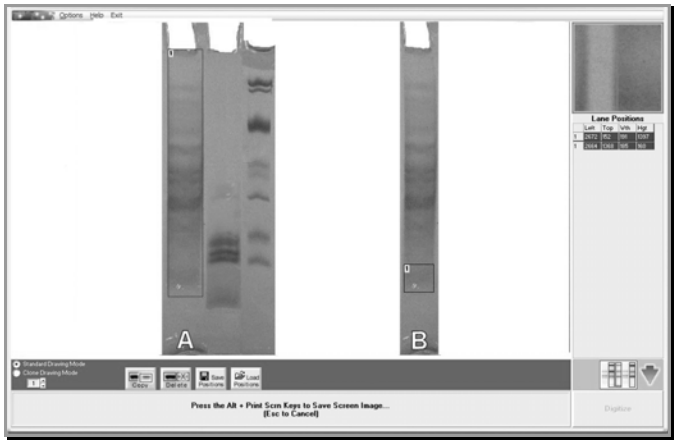


Fig. 4. Gel's scanning using UN-SCAN-IT software
(A) migration lane; (B) zone of the compounds of interest

CONCLUSION

Three different methods of processing for the juice of bitter melon fruits were acted: lyophilization drying, pulverization drying and vacuum drying. All methods led to powder outputs, with similar sensory characteristics, however spray-dried method led to a finer structure than the other two. SDS-PAGE electrophoretic analysis performed for separation of protein fractions and gel analysis for identification of studied protein fractions indicated the presence of the compounds of interest polypeptide-p and peptide MC6 in the inferior zone of lanes, both in standard juice sample and rehydrated dried juices too. That means drying process, regardless of method, did not damage studied hypoglycemic compounds, constituting a good preserving method. Dried product is recommended for therapeutic purposes in order to cause blood glucose level decrease: about 3 g fine powder of dried juice must be rehydrated for obtaining a daily dose of 50-60 ml.

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