International Food Research Journal 25(2): 842-847 (April 2018)
Journal homepage: http://www.ifrj.upm.edu.my

Total phenolic content of organic and conventional oranges and the effects of their juices on biochemical parameters of wistar rats

[1, 2 *]Dolinsky, M., [3]Fialho, E., [1]Souza, P. R., [1, 4]Ferreira-Fiochi, R. S., [1]Cardoso, L. M. F., [1, 4]Simões, V., [1]Maldronato, I. W., [1]Pimenta, N. M. A., [5]Ciarelli, G., [1]Barroso, S. G. and [1, 4]Rocha, G. S.

[1]Fluminense Federal University, Department of Nutrition and Dietetics, Rua Mário Santos Braga, nº 30, 4º andar, Valonguinho, Centro, Niterói – RJ, 24020-140, Brazil
[2]Fluminense Federal University, Graduate Program in Mother and Child Health, Rua Marquês do Paraná, 303 – 4º andar do prédio anexo ao HUAP, Centro, Niterói – RJ, 24030-210, Brazil
[3]Federal University of Rio de Janeiro, Basic and Experimental Nutrition Departament, Av. Carlos Chagas Filho 373, Centro de Ciências da Saúde – Instituto de Nutrição, Bloco J – subsolo, Sala J-06, Cidade Universitária - Ilha do Fundão, Rio de Janeiro – RJ, 21941-902, Brazil
[4]Fluminense Federal University, Graduate Program in Cardiovascular Sciences, Rua Marquês do Paraná, 303 – 4º andar do prédio anexo ao HUAP, Centro, Niterói – RJ, 24030-210, Brazil
[5]Oswaldo Cruz Foundation – FIOCRUZ, National School of Public Health Sérgio Arouca, Rua Leopoldo Bulhões 1480, Manguinhos, Rio de Janeiro - RJ, 21041-210, Brazil

Article history

Received: 21 December 2016
Received in revised form: 14 January 2017
Accepted: 15 January 2017

Keywords

Fruit and vegetables
Functional food
Organic food
Orange juice
Phenolic compounds

Abstract

This study investigated the effect of organic and conventional orange juice consumption on biochemical parameters of Wistar rats. Eighteen rats were divided into 3 groups: Control Group (CG), receiving a balanced diet and water; Organic Juice Group (OJG), receiving a balanced diet, water, and organic orange juice (15 mL/day), and; Conventional Juice Group (CJG), receiving a balanced diet, water, and conventional orange juice (15 mL/day). After 30 days, the animals were anesthetized and sacrificed. Blood was collected to obtain plasma for analysis. The Folin-Ciocalteu reagent method was used to determine the content of soluble polyphenols of the juices. Organic orange juice exhibited a significantly higher concentration of soluble polyphenols compared with conventional juice ($p<0.0001$). After the intervention, there were no statistical differences in glucose levels, total and LDL cholesterol, and triglycerides among the groups. There was a significant higher HDL-cholesterol in OJG compared to GC ($p=0.04$). It is suggested that the higher HDL levels in the group receiving the organic juice is related to the greater concentration of polyphenol found in our results.

Introduction

Fruits and vegetables are an important part of a balanced diet, since they not only provide a wide assortment of vitamins, minerals and phytochemicals, but also have fewer calories than other foods. Furthermore, fruits are a rich source of a variety of biologically active compounds called phytochemicals that can have complementary or overlapping mechanisms on lipid metabolism (Pereira *et al.,* 2014).

The most common phytochemicals in vegetables and fruits are flavonoids. They have antioxidant and anti-inflammatory properties that protect LDL from oxidation, preventing the development of atherosclerosis (Aptekmann and Cesar, 2010). Increasing scientific evidence shows that flavonoid consumption is associated with the reduction of low-grade inflammation and mortality for cardiovascular and neurodegenerative diseases, although the molecular mechanisms underlying such an effect are still to be clarified, since most of the data comes from animal and tissue culture testing (Aptekmann and Cesar, 2010; Cerletti *et al*., 2015). Previous studies have shown that flavonoids from citrus fruits, as orange, can reduce LDL-C and triglycerides in animals and humans, and increase HDL-C in hypercholesterolemic individuals (Aptekmann and Cesar, 2010; Hyson, 2015; Crowe-White *et al*., 2017). As already known, orange juice also has significant concentrations of vitamin C and folate, as well as potassium. Those levels of vitamin C could

*Corresponding author.
Email: *manudolinsky@gmail.com*

protect LDL and endothelial cells against intra- or extracellular oxidant stress and may reduce risk of atherosclerosis (Aptekmann and Cesar, 2010).

The term "organic" refers to the way agricultural products are grown and processed (Robinson *et al.*, 2016). It involves avoiding artificial fertilizers and pesticides, crop rotation, and other forms of husbandry to maintain soil fertility, weeds and diseases control (Bradbury *et al.*, 2014). Statista had shown that organic food sales have been growing in United States since 2000. In 2012, organic food sales generated 31.32 billion dollars in U.S. (Statista, 2015).

Organic foods nutrient content varies with the soil, weather conditions, and season. According to the literature, organic produce have significantly higher amounts of vitamin C, iron, magnesium and phosphorus when compared to non-organic counterparts. In addition to these micronutrients, organic produce have higher concentrations of phytochemicals (Worthington, 2004; Lairon, 2010; Crinnion, 2010; Hallmann, 2012). The aim of this study was to evaluate soluble polyphenol content in conventional and organic orange juice and the effects of organic versus conventional orange on lipid profile and glucose levels of Wistar rats.

Materials and Methods

Selection of samples

'Pera' sweet orange (*Citrus sinensis* (L.) Osbeck) is the most largely planted variety of citrus in Brazil, with more than 40% of the area cultivated with sweet oranges in that country (Salibe *et al.*, 2002; Cardoso *et al.,* 2010). For the experiment organic and conventional varieties of the fruit in natura and at commercial maturity stage were selected and purchased at one moment in a local market during their crop season (July-November) for a timely analysis.

Extraction of soluble polyphenols

The extraction of soluble polyphenol (SP) was performed according to the method described by Vinson *et al.* (2001). The SP extraction solution consisted of deionized water and methanol (1:1 v/v). Aliquots of 100 µl of orange juices were added to microtubes and combined with 500 µl of extraction solutions. The samples were placed in a water bath at 90°C for 3 hours. After this period, the samples were left at room temperature and the volume was filled up with absolute methanol to 1000 µl and centrifuged at 5000 rpm for 5 minutes. The supernatant, called polyphenol extract (PE), was used for the quantification of SP. The SP extract contains polyphenols mainly in the aglycone form and/or dissociated to cellular structures, being soluble in hydroalcoholic solutions.

Quantification of soluble polyphenols

The organic and conventional oranges were rinsed in cold running water, dried with paper towel and cut in half to obtain the juices. A centrifugal juicer (Walita Phillips®) was used to obtain 500 mL of juice from each type of orange. Immediately after the extraction the samples were stored at -22°C in a freezer (Sanyo® MDF-U537) until analysis.

The quantification of SP was conducted using the Folin–Ciocalteau's method, according to the methodology described by Karou *et al.* (2005), in which 75 µL of the Folin reagent was diluted in distilled water (1:1 v/v) and added to 30 µL of polyphenol extract and left at room temperature for 5 minutes in ELISA 96 well plates. Then, 75 µL of 20% sodium carbonate were added and kept at room temperature for 30 minutes in test tubes. The reading was made in spectrophotometer (Biochrom Anthos zenyth 200st microplate reader) at 750 nm. The SP content was expressed as milligrams of gallic acid equivalent per 100 milliliters of juice (mg GAE/100 mL). The Folin–Ciocalteau assay was performed twice, each in triplicate, and an average result taken.

Animals and diet

Eighteen adult (70-day-old) female Wistar albino rats (Rattus norvegicus), weighing 200-230 g, were housed individually in polypropylene cages in a controlled environment kept at 24ºC ± 2ºC, on a 12 h light/dark cycle (07:00-19:00 h). The study protocol was approved by the Ethics Committee for Animal Experimentation of the Federal Fluminense University (protocol number CEUA 507/2014) and was performed according to its guidelines. The animals were divided into 3 groups of 6 each: Control Group (CG), receiving a balanced diet and water; Organic Juice Group (OJG), receiving a balanced diet, water and organic orange juice (15 mL/day), and; Conventional Juice Group (CJG), receiving a balanced diet, water and conventional orange juice (15 mL/day). Standard diet and water were provided ad libitum. The diet was prepared according to AIN-93M recommendations and the orange juice dosage was calculated based on the observation of the average liquid consumption (mL/rat/day) of the group. Juice consumption was monitored daily in order to guarantee that all subjects were ingesting the whole dosage. Food intake and body weights were monitored weekly.

Data and Sample Collection

The experiment was terminated after 30 days, at which point all rats were anaesthetized and sacrificed. Overnight fasting blood was collected into a heparinized tube after the animals were killed, in order to obtain plasma samples for analyses.

Biochemical blood analysis

Blood glucose, triglycerides, total cholesterol, HDL and LDL fractions were measured using commercial assay kits (Bioclin, Belo Horizonte, Brazil), as in Jatsa *et al*. (2016) and Bernardes *et al.* (2016), among others.

Statistical analysis

Values were expressed as mean ± standard deviation (SD). Student's t-test or Mann-Whitney test was used whenever appropriate for comparison of means between organic and conventional fruits. For comparison of means between groups, data were analyzed by one-way analysis of variance (ANOVA) with Duncan's post-test. The GraphPad Prism 5.0 software was used to conduct the analyses and significance was considered at $p<0.001$.

Results

Organic orange juice was found to have a significantly higher concentration of total polyphenols compared to conventional juice (Table 1). During the study, there were no significant differences in weight gain (Figure 1A), food intake (Figure 1B) or juice consumption (Figure 1C) among groups. Finally, as shown in Table 2 there were no statistical differences in glucose, total cholesterol, LDL and triglycerides levels among groups. However, there was a significantly higher HDL level in OJG in comparison to the CG.

Table 1. Soluble polyphenol content in organic (OOJ) and conventional orange juices (COJ).

	OOJ	COJ	P Value
Soluble polyphenols	37.15 ± 1.54	17.29 ± 0.36	0.0002

The values are expressed as mg GAE/100mL of juice and shown as averages ± SD. (*) $p < 0.05$.

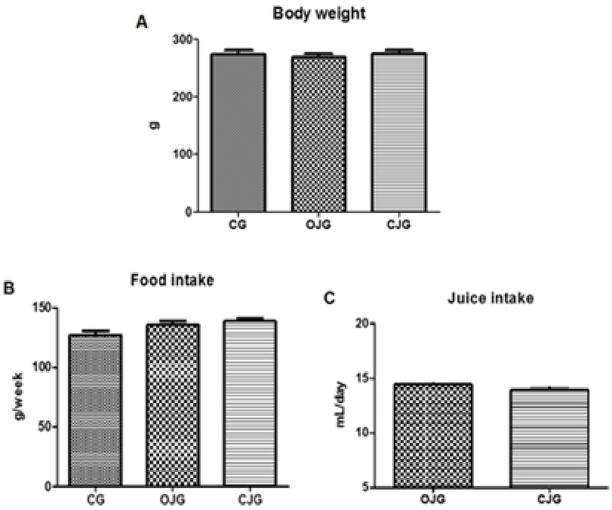

Figure 1. Body weight (A), food intake (B) and juice intake (C) in control group (CG), organic juice group (OJG) and conventional juice group (CJG). The values are shown as averages ± SD.

Discussion

Nowadays organic farming is presented in the literature as an economically sustainable venture, capable of contributing to the development of small farmers (Delbridge *et al*., 2011), besides being also a way of minimizing food production exposure to carcinogens (Mendoza *et al*., 2004; Helmfrid *et al*., 2012; Nath *et al.*, 2013; Wallace, 2015). The results obtained in this study indicate a significantly higher content of polyphenols in organic orange juice in comparison to conventional orange juice ($p<0.001$). Johansson and colleagues (2014) suggest that this high polyphenol content found in organic farming systems is related to the response to pathogens, as a form of natural plant defense.

Regarding polyphenol content in conventional fruits and vegetables, Faller and Fialho (2009) observed in a group of 12 food items that ponkans *(Citrus reticulate)* yielded the highest contend, followed by bananas *(Musa acuminata),* and oranges *(Citrus sinensis)*. The results furthermore corroborate the findings of Buena *et al.* (2014) and Dani *et al*. (2007) when compared the content of total polyphenols in different varieties of grapes grown in organic and conventional system. Dani *et al.* (2007) also observed that the grapes from organic farms have higher antioxidant capacity compared to conventional grapes.

Oliveira *et al*. (2013) noted an increase in the amount of the enzyme phenylalanine ammonia lyase, an enzyme involved in flavonoids biosynthesis in organic tomatoes, which may explain the significantly higher content of polyphenols in this group of food compared to conventional tomatoes. These data suggest that organic farming has the ability of modifying transcripts or enzymes activities levels of controlling intermediary steps of phenolic compounds biosynthetic pathway.

Despite the food and drinks have been offered ad libitum during the experiment, there was no

Table 2. Biochemical analysis of Control Group (CG), Organic Juice Group (OJG) and Conventional Juice Group (CJG).

	CG	OJG	CJG	P Value
Glucose	157.3 ± 16.43	160.3 ± 8.55	143.7 ± 14.39	0.09
Total Cholesterol	77.0 ± 12.07	84.0 ± 8.08	84.33 ± 16.49	0.54
HDL	30.50 ± 2.88	35.33 ± 2.73	34.50 ± 3.89	0.04*
LDL	34.80 ± 12.18	36.77 ± 8.36	39.23 ± 13.80	0.81
Triglycerides	58.50 ± 24.74	62.83 ± 8.70	53.00 ± 9.90	0.58

All parameters are expressed in mg/dL. The values are shown as averages ± SD. (*) $p < 0.05$ when compared with the control group (CG).

significant difference in their consumption, and hence, no differences in final body weight. The analysis of glucose concentration is presented in Table 2. As can be seen, no significant differences between the groups were found. Dong *et al.* (2016), in a study comparing different orange juices effects on blood glucose and insulin levels after a high-fat diet supply, observed a better glycemic control and postprandial insulinemia after consumption of the juice with higher fiber amount (plus the pulp of the orange). On the other side, in the present study the drinks were whole and made moments before offering to the animals, what could have yielded, or at least, contributed to, the different results, since we found no differences on those parameters, compared to CG. On the other hand, all animals received a balanced diet and were healthy, so it was not expected any glucose metabolism disregulation.

Concerning total cholesterol concentration, there was no significant difference among groups, although hesperidins, a component present in all orange varieties, have a known hypocholesterolemic effect (Morand *et al*., 2011). Unfortunately, in the present study there was no data regarding this component. However, the OJG showed significantly higher levels of HDL ($p<0.05$) than CG, but no difference compared to CJG. Kurowska *et al.* (2000), in a study with humans, found higher levels of HDL in the group that received orange juice, when compared to the control group. These results are similar to the present ones, where groups receiving orange juice had higher HDL levels. In another study, Baba *et al*. (2007) suggest that content of total polyphenols contributes not only to increase in HDL, but also LDL oxidation prevention, reinforcing polyphenols importance in a cardioprotective diet. Higher HDL levels found in organic juice group may be related to highest concentration of polyphenol found in organic orange. Notwithstanding, orange juice, organic and conventional as well as control group did not display any differences concerning LDL levels.

No differences were observed on triglycerides in the present study. Cerletti *et al.* (2015) observed reduction on triglycerides after fat diet and orange juice consumption, as a result of hesperidins effects on hormone sensitive lipase action. Those contradictory results may be a result of differences in diet composition. Finally, we must underline a relevant limitation of the study that must be overcome before we can further generalize its results: since all animals used were from the start healthy, with a balanced diet, it is possible that a larger time span – and, thus, a larger exposure to the organic and conventional juices – could be necessary in order to reinforce their effect and make the results more robust.

Conclusion

In conclusion, results show the importance of encouraging orange juice consumption, especially organic, due to its high polyphenol content compared to conventional orange juice. Furthermore, data suggest a possible cardioprotective effect due to higher HDL levels.

Acknowledgement

The authors would like to thank the Research Foundation of the State of Rio de Janeiro (FAPERJ) and the Pro-Rectory of Research, Graduate Studies and Innovation of the Fluminense Federal University (PROP – UFF) for supporting this research.

References

Aptekmann, N. P. and Cesar, T. B. 2010. Orange juice improved lipid profile and blood lactate of overweight middle-aged women subjected to aerobic training. Maturitas 67(4): 343-347.

Baba, S., Osakabe, N., Kato, Y., Natsume, M., Yasuda, A., Kido, T., Fukuda, K., Muto, Y. and Kondo, K. 2007. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. The American Journal of Clinical Nutrition 85(3): 709-717.

Bradbury, K. E., Balkwill, A., Spencer, E. A., Roddam, A. W., Reeves, G. K., Green, J., Key, T. J., Beral, V. and Pirie, K. 2014. Organic food consumption and the incidence of cancer in a large prospective study of women in the United Kingdom. British Journal of Cancer 110(9): 2321-2326.

Bunea, C. I., Pop, N., Babeş, A. C., Matea, C., Dulf, F.

V. and Bunea, A. 2012. Carotenoids, total polyphenols and antioxidant activity of grapes *(Vitis vinifera)* cultivated in organic and conventional systems. Chemistry Central Journal 6(1): 1-9.

Cardoso, S. C., Barbosa-Mendes, J. M., Boscariol-Camargo, R. L., Christiano, R. S. C., Bergamin Filho, A., Vieira, M. L. C., Mendes, B. M. J. and Mourão Filho, F. D. A. A. 2010. Transgenic sweet orange (*Citrus sinensis* L. Osbeck) expressing the attacin A gene for resistance to *Xanthomonas citri s*ubsp. citri. Plant Molecular Biology Reporter 28(2): 185-192.

Cerletti, C., Gianfagna, F., Tamburrelli, C., De Curtis, A., D'Imperio, M., Coletta, W., Giordano, L., Lorenzet, R., Rapisarda, P., Recupero, G. R. and Rotilio, D. 2015. Orange juice intake during a fatty meal consumption reduces the postprandial low-grade inflammatory response in healthy subjects. Thrombosis Research 135(2): 255-259.

Crinnion, W. J. 2010. Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. Alternative Medicine Review 15(1): 4-12.

Crowe-White, K., Parrott, J.S., Stote, K.S., Gutschall, M., Benson-Davies, S., Droke, E., O'Neil, C.E., Wolfram, T. and Ziegler, P. 2017. Metabolic impact of 100% fruit juice consumption on antioxidant/oxidant status and lipid profiles of adults: an evidence-based review. Critical Reviews in Food Science and Nutrition 57(1): 152-162.

Dani, C., Oliboni, L. S., Vanderlinde, R., Bonatto, D., Salvador, M. and Henriques, J. A. P. 2007. Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food and Chemical Toxicology 45(12): 2574-2580.

Delbridge, T. A., Coulter, J. A., King, R. P., Sheaffer, C. C. and Wyse, D. L. 2011. Economic performance of long-term organic and conventional cropping systems in Minnesota. Agronomy Journal 103(5): 1372-1382.

Dong, H., Rendeiro, C., Kristek, A., Sargent, L. J., Saunders, C., Harkness, L., Rowland, I., Jackson, K. G., Spencer, J. P. E. and Lovegrove, J. A. 2016. Addition of orange pomace to orange juice attenuates the increases in peak glucose and insulin concentrations after sequential meal ingestion in men with elevated cardiometabolic risk. The Journal of Nutrition 146(6): 1197-1203.

Faller, A. L. K. and Fialho, E. 2009. Polyphenol availability in fruits and vegetables consumed in Brazil [in Portuguese]. Revista de Saúde Pública 43(2): 211-218.

Hallmann, E. 2012. The influence of organic and conventional cultivation systems on the nutritional value and content of bioactive compounds in selected tomato types. Journal of the Science of Food and Agriculture 92(14): 2840-2848.

Helmfrid, I., Berglund, M., Löfman, O. and Wingren, G. 2012. Health effects and exposure to polychlorinated biphenyls (PCBs) and metals in a contaminated community. Environment International 44: 53-58.

Hyson, D. A. 2015. A review and critical analysis of the scientific literature related to 100% fruit juice and human health. Advances in Nutrition: An International Review Journal 6(1): 37-51.

Jatsa, H. B., Russo, R. C., de Jesus Pereira, C. A., Aguilar, E. C., Garcia, C. C., Araújo, E. S., Oliveira, J. L. R., Rodrigues, V. F., de Oliveira, V. G., Alvarez-Leite, J. I. and Braga, F. C. 2016. Improvement of the liver pathology by the aqueous extract and the n-butanol fraction of Sida pilosa Retz in Schistosoma mansoni-infected mice. Journal of Ethnopharmacology 180: 114-123.

Johansson, E., Hussain, A., Kuktaite, R., Andersson, S. C. and Olsson, M. E. 2014. Contribution of organically grown crops to human health. International Journal of Environmental Research and Public Health 11(4): 3870-3893.

Karou, D., Dicko, H. M., Simpore, J. and Traore, A. S. 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of *Burkina Faso.* African Journal of Biotechnology 4(8): 823-828.

Kurowska, E. M., Spence, J. D., Jordan, J., Wetmore, S., Freeman, D. J., Piché, L. A. and Serratore, P. 2000. HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. The American Journal of Clinical Nutrition 72(5): 1095-1100.

Lairon, D. 2010. Nutritional quality and safety of organic food. Agronomy for Sustainable Development 30(1): 33-41.

Mendoza, B. J., Alonso, F. M. U., Díaz, Z. S., Beltrán, L. T. and Velázquez, O. V. 2004. Food safety in rabbit meat production. In Proceedings of the 8th World Rabbit Congress, p. 589-596. Puebla: World Rabbit Science Association. Retrieved on May 15, 2016 from Proceedings of the 8th World Rabbit Congress Website:*https://world-rabbit-science.com/WRSA-Proceedings/Congress-2004-Puebla/Puebla-2004-a.htm*

Morand C., Dubray C., Milenkovic D., Lioger D., Martin J. F., Scalbert A. and Mazur A. 2011. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. The American Journal of Clinical Nutrition 93: 73–80.

Nath, A., Vendan, S. E., Priyanka, J. K. S., Singh, C. K. and Kumar, S. 2013. Carcinogenic pesticides residue detection in cow milk and water samples from Patna, India. Current Trends in Biotechnology and Chemical Research January-June 3(1): 1-7.

Oliveira, A. B., Moura, C. F. H., Gomes-Filho, E., Marco, C. A., Urban, L., and Miranda, M. R. A. 2013. The impact of organic farming on quality of tomatoes is associated to increased oxidative stress during fruit development. PLoS ONE 8(2): e56354.

Pereira, A. C., Dionísio, A. P., Wurlitzer, N. J., Alves, R. E., de Brito, E. S., Silva, A. M. D. O., Brasil, I. M. and Mancini Filho, J. 2014. Effect of antioxidant potential of tropical fruit juices on antioxidant enzyme profiles and lipid peroxidation in rats. Food Chemistry 157: 179-185.

Robinson, L., Segal, J. and Segal, R. 2016. Organic foods: all you need to know. Retrieved on May 15, 2016 from HelpGuide Website: www.helpguide.org/articles/healthy-eating/organic-foods.htm

Statista Inc. 2015. U.S. Organic Food Industry - Statistics and Facts. Retrieved on May 15, 2016 from Statista Website:*http://www.statista.com/topics/1047/organic-food-industry*

Salibe, A. A., Teófilo Sobrinho, J. and Müller, G. W. 2002. An overview about pera sweet orange knowledge [in Portuguese]. Laranja 23(1): 231-245.

Vinson, J. A., Su, X., Zubik, L. and Bose, P. 2001. Phenol antioxidant quantity and quality in foods: fruits. Journal of Agricultural and Food Chemistry 49(11): 5315-5321.

Wallace, D. R. 2015. Environmental pesticides and heavy metals – role in breast cancer. In Larramendy, M. L. and Soloneski, S. (Eds). Toxicity and Hazard of Agrochemicals, p. 39-70. Rijeka: InTech.

Worthington, V. 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. The Journal of Alternative and Complementary Medicine 7(2): 161-173.