Biological Forum – An International Journal **16(1): 161-165(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Manufacture and characterization of Low Cholesterol Paneer Prepared from Buffalo Milk

Reji A.J.[1*], Divya M.P.[2], Rajakumar S.N.[3], Rejeesh R.[4] and A.K. Beena[5]

[1]M.Tech. Scholar, Department of Dairy Chemistry, Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, Thrissur under Kerala Veterinary and Animal Sciences University (Kerala), India.
[2]Assistant Professor, Department of Dairy Chemistry, Verghese Kurien Institute of Dairy and Food Technology, Kerala Veterinary and Animal Sciences University (Kerala), India.
[3]Professor and Head, Department of Dairy Technology, Verghese Kurien Institute of Dairy and Food Technology, Kerala Veterinary and Animal Sciences University (Kerala), India.
[4]Assistant Professor, Department of Dairy Microbiology, College of Dairy Science and Technology, Trivandrum under Kerala Veterinary and Animal Sciences University (Kerala), India.
[5]Professor and Head, Department of Dairy Microbiology, Verghese Kurien Institute of Dairy and Food Technology, Kerala Veterinary and Animal Sciences University (Kerala), India.

(Corresponding author: Reji A.J.)*

(Received: 20 November 2023; Revised: 29 November 2023; Accepted: 26 December 2023; Published: 15 January 2024)
(Published by Research Trend)

ABSTRACT: Raw milk contains 0.25–0.40% of its total lipids as cholesterol, which has been linked chiefly to the fat globular membrane and the milk proteins. As a result, dairy products that are high in fat have a high cholesterol level. High cholesterol diets may lead to various coronary heart diseases and there is scope for methodologies that could reduce cholesterol in foods. Beta cyclodextrin (β-CD) is a cyclic oligosaccharide and due to its specific shape can entrap cholesterol molecule effectively. It is inexpensive and has been classified as safe by the Joint Expert Committee on Food Additives (JECFA) and the World Health Organization. Cholesterol removal by application of β-CD was highly selective, without adversely affecting the nutritional and flavour properties. In this study buffalo milk was treated with varying amounts of beta cyclodextrin (0.6,0.8 and 1%) and paneer was made. A control paneer was also prepared under identical conditions, except for the beta cyclodextrin treatment, and was compared to the treatments. The paneer treated with 1% beta cyclodextrin showed maximum cholesterol lowering potential. Cholesterol estimation was performed using enzymatic diagnostic kit. The paneer and whey were analyzed for residual beta cyclodextrin content and detected the same at an increasing trend as the rate of addition of beta cyclodextrin to the buffalo milk was increased. The physico-chemical properties of the different treatments were compared to those of control.

Keywords: Low cholesterol paneer, cholesterol, beta cyclodextrin.

INTRODUCTION

Paneer is one of the widely used and popular indigenous dairy products prepared by the heat and acid coagulation of milk. It is thought that the nomads of south west Asia were the first, to develop several distinctive heat and acid coagulated varieties of cheese (Mathur *et al*., 1986), as there is documental evidence suggesting that the people of Kusana and Saka Satavahana periods (AD 75- 300) used to consume the solid mass prepared from mixture of warm milk and curd, which seems to be the earliest version of today's heat and acid-coagulated milk products like paneer (Mathur *et al*., 1986). Paneer Khiki is one of the Iranian nomadic cheeses, developed by Bakhtiari tribe of Iran. The nomads of Afghanistan developed white paneer which was a staple food for them, they developed two distinct varieties of paneer, when made from raw milk, it is known as paneer-e-kham, and from boiled milk, paneer-e-pokhta. Persian and Afghan invaders probably brought paneer to India first, which is the most likely reason for its wide popularity in the North Western parts of India and Southern regions of Jammu and Kashmir due to the influence of foreign settlers in these regions. Nonetheless, paneer has only become widespread in other parts of India during the last 50 years, most likely as a result of widespread migration from one area to another. It is described by the Food Safety and Standards Authority of India as 'the product obtained from any variant of milk with or without added milk solids, by precipitation with permitted acidulants and heating'. The regulations also mandate a maximum moisture percentage (m/m) of 60.0 and a minimum milk fat percentage (dry matter basis) of 50.0. The nutty flavor and marble white color of paneer, which is prepared from buffalo milk, are its defining features (Aneja *et al*., 2002). Owing to its high fat and protein content and plentiful mineral content (such as calcium and phosphorus), this native product is considered quite vital when it comes to diet, particularly when considering Indian vegetarian food. Nevertheless, paneer has a relatively short shelf life (Kanawjia and Singh 2000). Paneer loses its chemical and microbiological quality after just one day at ambient temperature and six days in the refrigerator.

In milk, cholesterol is attached to the fat globular membrane, fat core, and proteins in the serum portion. It makes up 0.25–0.45% of the total lipids in milk (Schlimme and Kiel 1990). Any event causing a significant disruption of the fat globular membrane will result in the passing of cholesterol to the aqueous phase. Butter milk have 9 times higher cholesterol per gram of fat than butter (Tylkin *et al*., 1975). Most of the cholesterol in milk fat exists as free cholesterol (85-90%) and a minor portion exists in esterified form (10-15%). As per Fuke and Matsuoka (1974) cheese was found to contain 69.3 ng/100g of average cholesterol content.

Various techniques have been devised for removing cholesterol in order to lower the cholesterol level in food products. These include steam stripping, molecular distillation, reaction with cyclic anhydride, treatments with adsorbents like saponin, activated charcoal or cyclodextrin and enzymatic treatments. Steam stripping is a process similar to that used in the deodorization of vegetable oils. Here the milk fat is first deaerated under vacuum after which it is heated with steam up to 232°C and then subjected to steam at low pressure in cylindrical tall chamber. The anhydrous milk fat passing over a series of plates is spread in thin layers, which increases the efficiency of stripping. The steam rises and carries with it the evaporated cholesterol to be condensed and collected with other volatiles. 93% of the cholesterol may be eliminated by this method (Schlimme and Kiel 1990). In molecular distillation anhydrous milk fat is molecularly distilled at temperature 190 and 210°C at vacuum of 10-4 Torr. More than 93% of the total cholesterol was present in the fractions distilled at 190 and 210°C, which corresponded to 3.43 and 3.99% of the starting mass, respectively (Lanzani *et al*., 1994). Reaction with cyclic anhydride method is based on the reaction between the hydroxyl group of cholesterol and a cyclic anhydride such as succinic anhydride. It is possible to extract cholesterol from lipids using an aqueous alkali due to its conversion into an acid derivative. Addition of acetic acid increases the rate of reaction and prevents the distillation of cyclic anhydride from reaction mixture (Gu *et al*., 1994). Adsorption methods is based on the principle that cholesterol can be removed by its adsorption on certain material. The adsorbents maybe activated charcoal, saponins or β-cyclodextrin. According to Bindal and Jain (1973) half of the cholesterol present in milk fat can be removed through treatment of liquid fat with activated charcoal. Lee *et al*. (1999); Sharma *et al*. (1999) studied the use of saponins for cholesterol removal milk fat. Enzymatic process of cholesterol removal involves the conversion of cholesterol to biologically inactive non -toxic, non absorbable products like corposterol which is poorly adsorbed the body using enzymes like cholesterol reductase (Macdonald *et al*., 1983).

Recently, β-cyclodextrin (a starch hydrolysed product) has been effectively used for cholesterol removal from dairy products, lard and egg-yolk. β-cyclodextrin is found to be non-toxic, non hygroscopic, chemically stable and edible. The selective elimination of cholesterol can be achieved through the use of β-cyclodextrin (β-CD), without appreciably affecting the amount of other nutritional and taste components (Kolaric *et al*., 2022). β-CD is a doughnut-shaped cyclic oligosaccharide made up of seven glucose units. Because of its appropriate diameter, it may nearly perfectly enclose a cholesterol molecule (Alonso *et al*., 2009). β-CD is inexpensive and has been classified as ‘safe’ by the Joint Expert Committee on Food Additives (JECFA) and the World Health Organization (Alonso *et al*., 2009). β-CD may remove cholesterol from milk and dairy products with excellent efficiency—up to 98% (Kolaric *et al*., 2022).

MATERIALS AND METHODS

Milk: the buffalo milk used for paneer preparation was procured from university Dairy plant, Mannuthy, Thrissur. β Cyclodextrin 97% pure (Food grade) was purchased from RP Chemicals, Issaji Street Masjid Bandar West, Mumbai. Citric Acid: Food grade citric acid was purchased from local market, Thrissur. Enzymatic diagnostic kit for Cholesterol: purchased from Arkray Healthcare Private Limited Surat, Gujarat. Kit consisted of Cholesterol mono reagent, Cholesterol standard (200mg/dL). Other Chemicals: Analytical grade chemicals were procured from approved firms

Selection of rate of addition of β-cyclodextrin for the preparation of low cholesterol paneer: Milk used for paneer preparation was treated with 0.6, 0.8, and 1% of β-cyclodextrin and the treatment with highest cholesterol removal potential was selected.

Procedure: The following sequence was followed while performing the experiment.

Standardization

Buffalo milk was standardized to 5% fat.

β Cyclodextrin Treatment. Cholesterol removal by β-CD was performed according to Kolaric and Simko (2022). For the preparation of the milk used for the production of paneer, the following treatment was applied: 1 L of pasteurized milk (5 % fat content) was mixed with 3 different levels of β-CD (0.6, 0.8, and 1% w/vol) at 840 rpm, 25°C and for 20 min using a magnetic stirrer. Vigorous stirring using a ladle was performed for 10 minutes. This was supposed to cause enough disruption of the fat globular membrane. The milk was then settled for 12 hours at 4 ◦C and finally again vigorously stirred for 10 minutes using a ladle till heating, for the preparation of paneer.

Preparation of Paneer. Paneer was prepared using treated milk as per the procedure by Bhattacharya *et al*. (1971).

Estimation of Cholesterol content. Cholesterol estimation viaenzymatic diagnostic kit was performed according to Parmar *et al*. (2016) for the prepared paneer samples.

Assay Principle for Total Cholesterol. Cholesterol esters are hydrolyzed by cholesterol esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase (CHOD) oxidizes the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide couples with 4-Aminoantipyrine (4AAP) and phenol to produce red Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm (490-510) and is proportional to amount of total cholesterol concentration in the sample.

$$\text{Cholesterol esters} \xrightarrow{\text{CE}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-En-3-One} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\text{POD}} \text{Quinoneomine Dye} + H_2O_2$$

Procedure. Protocol for cholesterol estimation in sample using enzymatic diagnostic kit was performed according to Parmer *et al*. (2016). In order to estimate the cholesterol level in paneer, a sample of 0.2 grams of paneer was taken.

RESULT AND DISCUSSION

A. Cholesterol removal potency of β-cyclodextrin

The cholesterol removal potential of different concentrations of β-cyclodextrin treatments to raw buffalo milk are given in the table below. The cholesterol content of each sample was ascertained using the cholesterol estimation assay. Fresh paneer samples was used for the analysis.

Table 1: Cholesterol removal potential of β-cyclodextrin

	control	T1	T2	T3	F value
Cholesterol content in paneer (mg/100g)	43.29 ± 2.01^{a}	30.64 ± 2.73^{b}	17.76 ± 2.76^{c}	6.68 ± 1.59^{d}	46.44**
cholesterol removal from paneer (%)	0.00 ± 0.00^{a}	28.65 ± 7.84^{b}	58.41 ± 7.39^{c}	84.17 ± 4.16^{d}	39.84**

Figures are mean ± standard error of four replications, *-Significant at five per cent level (p<0.05) **-Significant at one per cent level (p<0.01), ns-non-significant (p> 0.05), a-d -Means with different superscript vary significantly within a column.

Because paneer made from buffalo milk treated with 1% β-cyclodextrin had the lowest cholesterol levels, it was determined that this degree of treatment was ideal for making functional paneer. The cholesterol content of paneer prepared from buffalo milk treated with 0.6, 0.8 and 1% of β-cyclodextrin were 30.64, 17.76, and 6.69 mg/100g respectively. The cholesterol content of control paneer was 41.29 mg/100g. From this we can calculate the cholesterol removal per cent in each treatment which obtained as 28.65, 58.41 and 84.17% for buffalo milk treated with 0.6, 0.8 and 1% of β-cyclodextrin. This study demonstrated a significant increase in cholesterol removal at 1% β-cyclodextrin levels. According to Kolaric *et al*. (2022), treatment of cow milk and cream with 1% and 5% β-cyclodextrin was effective in cholesterol removal of 97.3% and 95.6%, respectively. The cholesterol content in butter was reduced to 95.6%, in cottage cheese to 97.9%, and in soft cheese to 97.7% with 5%, 1% and 2.5 % added β-cyclodextrin. According to Alonso *et al*. (2009) approximately 97.6% cholesterol reduction was observed in ewe's Milk Manchego cheese that was treated using β-cyclodextrin. The addition of 0.4, 0.6, 0.8, and 1.0% β-CD to the milk removed from 65.4 (±0.61) to 95.3 (±0.37) % of cholesterol at 4°C in 20 min. Higher than 0.8% concentrations of β-cyclodextrin did not seem to improve the efficiency of eliminating cholesterol.

B. Physico-chemical properties

The physico-chemical properties and yield of control and treatment paneer samples is given in the following table. The physico-chemical properties were found using the prescribed methods of FSSAI. Fresh paneer was used for the analysis. The fat % and yield of control and different treatments are given in the subsequent figures. The findings revealed no noticeable differences in composition between the various treatments and the control.

Table 2: Yield and Physico-chemical properties of samples

	Control	T_1	T_2	T_3	F value
Yield (%)	19.55±0.46	19.62±0.41	19.87±0.87	19.74±0.62	0.05^{ns}
Moisture (%)	56.12±.91	54.16±1.00	55.87±1.07	56.31±0.69	1.07^{ns}
Fat on DM Basis (%)	56.35±1.20	56.15±3.44	55.48±1.96	58.92±2.54	0.77^{ns}
Protein (%by mass)	22.47±0.49	22.40±0.51	21.73±0.33	22.57±0.72	0.59^{ns}
TA(%LA)	0.18±0.01	0.18±0.01	0.18±0.00	0.18±0.00	0.054^{ns}

Figures are mean ± standard error of four replications, ns-non-significant (p> 0.05),

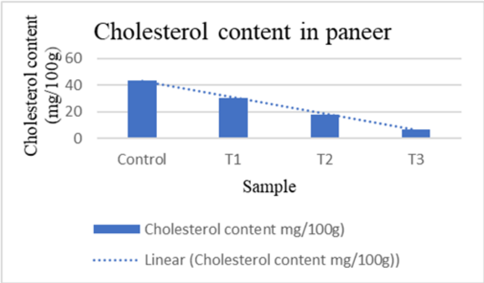

Fig. 1. Cholesterol content in control and different treatments.

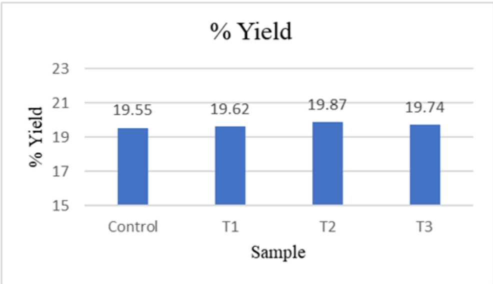

Fig. 2. Yield of control and different treatments.

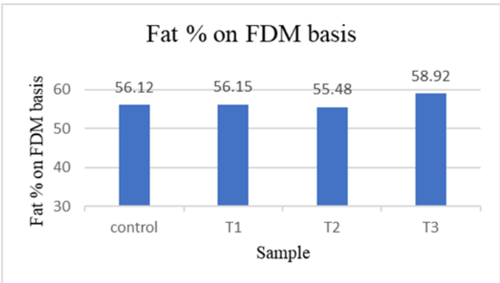

Fig. 3. Fat % of control and different treatments.

Yield of paneer prepared from buffalo milk treated with 0.6, 0.8 and 1% of β-cyclodextrin were 19.62, 19.87 and 17.74 % respectively. The yield of control paneer was 19.55%. There was no significant difference between the yields; hence, it can be inferred that the level of addition of β-cyclodextrin has no effect on yield. The moisture content of paneer prepared from buffalo milk treated with 0.6, 0.8 and 1% of β-cyclodextrin were 54.16, 55.87 and 56.31 % respectively. The moisture content of control paneer was 56.12%. There was no significant difference between the moisture content; hence, it can be inferred that the level of addition of β-cyclodextrin has no effect on moisture content. The fat content (on dry matter basis) of paneer prepared from buffalo milk treated with 0.6, 0.8 and 1% of β-cyclodextrin were 56.15, 55.18 and 58.92 % respectively. The fat content of control paneer was 56.35%. There was no significant difference between the fat content; hence, it can be inferred that the level of addition of β- cyclodextrin has no effect on fat content. The protein content of paneer prepared from buffalo milk treated with 0.6, 0.8 and 1% of β-cyclodextrin were 22.40, 21.73 and 22.57 % respectively. The protein content of control paneer was 22.47%. There was no significant difference between the protein content; hence, it can be inferred that the level of addition of β-cyclodextrin has no effect on protein content. The acidity of paneer prepared from buffalo milk treated with three levels of β-cyclodextrin and control paneer was obtained at 0.18%.; hence, it can be inferred that the level of addition of β-cyclodextrin has no effect on protein content. These results are in accordance with the findings of Elwahsh (2018), who reported that the yield and main chemical components of cholesterol-reduced cheese using β-cyclodextrin and regular cream cheese were almost similar. But during storage, some variations in moisture, acidity, acid value, and pH were documented. According to Ha *et al*. (2010). The content of lactose in the control cream (without the treatment by β-cyclodextrin) was 2.74%, and the amounts of lactose entrapped by β-cyclodextrin ranged from 0.00 to 0.03%. The total amounts of the entrapped short-chain free fatty acid (FFA) and free amino acid (FAA) ranged from 0.04 to 0.12 ppm and from 0.37 to 0.48 μmol⁄mL, respectively. The amounts of the entrapped water-soluble vitamins (L-ascorbic acid, niacin, thiamine and riboflavin) ranged from 0.01 to 0.05 ppm, from 0.01 to 0.05 ppm, from 0.00 to 0.04 ppm and from 0.02 to 0.05 ppm, respectively. The entrapped amounts of nutrients mentioned above were not remarkably affected by the concentrations of crosslinked β-cyclodextrin (4–12%, w⁄v). The very small amounts of residual β-cyclodextrin in the cholesterol-removed cream were measured (1.86–6.11 ppm). Nguyen *et al*. (2021) demonstrated that the fatty acid profile of cream was not impacted by the cholesterol removal using β-cyclodextrin. Bhatia *et al*. (2019) studied properties of low cholesterol ghee and reported that composition of both control and low-cholesterol ghee were almost similar**.**

CONCLUSION

Low cholesterol paneer was prepared from buffalo milk with the addition of β cyclodextrin. It was found that the addition of 1 % of the same had the highest cholesterol removal potential. There was no noticeable difference in the Yield and Physico-Chemical characteristics between the control and low-cholesterol produced paneer.

FUTURE SCOPE

The development of dairy products with low cholesterol can be essential in today's diet to lower the risk of CVD. Also, there is an enormous demand for them. The method of using β-cyclodextrin to lower the cholesterol content is very economical and easy. It opens a wide window for a range of low cholesterol dairy products.

REFERENCES

Alonso, L., Cuesta, P., Fontecha, J., Juarez, M. and Gilliland, S. E. (2009). Use of β-cyclodextrin to decrease the level of cholesterol in milk fat. *Journal of Dairy Science, 92*(3), 863-869.

Aneja, R. P., Mathur, B. N., Chandan, R. C. and Baneerjee, A. K. (2002). Technology of Indian milk products. *Dairy India Publication, Delhi.* 133–158.

Bhatia, P., Sharma, V., Arora, S. and Rao, P. S. (2019). Effect of cholesterol removal on compositional and the physicochemical characteristics of anhydrous cow milk fat (cow ghee). *International Journal of Food Properties, 22*(1), 1-8.

Bhattacharya, D. C., Mathur, O. N., Srinivasan, M. R. and Samlik, O. (1971). Studies on the method of production and shelf-life of paneer (cooking type acid coagulated cottage cheese). *Journal of Food Science and Technology, 8*(5), 117-121.

Bindal, M. P. and Jain, M. K. (1973). Studies on cholesterol content of cow and buffalo ghee. *Indian Journal of Animal Sciences, 43*, 918–924.

Elwahsh, N. A. (2018). Manufacture of Cholesterol-Reduced Double Cream Cheese from Buffalo's Milk. *Journal of food and dairy sciences, 9*(12), 395-398.

Fuke, Y. And Matsuka, H. (1974). Cholesterol content and identification of foreign fats in processed cheese. *Journal of Japan Society of Nutrition and Food Science, 27*, 269.

Gu, Y. F., Chen, Y and Hammond, E. G. (1994). Use of cyclic anhydrides to remove cholesterol and other hydroxy compounds from fats and oils. *Journal of the American Oil Chemists' Society*, *71*(11), 1205-1209.

Ha, H. J., Lee, J. E., Chang, Y. H and Kwak, H. S. (2010). Entrapment of nutrients during cholesterol removal from cream by crosslinked β-cyclodextrin. *International Journal of Dairy Technology, 63*(1), 119-126.

Kanawjia, S. K. and Singh, S. (2000). Technological advances in paneer making. *Indian Dairyman*, *52*(10), 45-50.

Kolaric, L., Kantorova, P. and Simko, P. (2022). β-Cyclodextrin as the Key Issue in Production of Acceptable Low-Cholesterol Dairy Products. *Molecules*, *27* (9), 2919- 2926.

Kolaric, L. and Simko, P. (2022). Effect of processing conditions on measure of cholesterol removal from milk and cream. *Monatshefte für Chemie-Chemical Monthly, 153*(11), 1069-1075.

Lanzani, A., Bondioli, P., Mariani, C., Folegatti, L., Venturini, S., Fedeli, E. and Barreteau, P.(1994). A new short-path distillation system applied to the reduction of cholesterol in butter and lard. *Journal of the American Oil Chemists' Society*, *71*(6), 609-614.

Lee, D. K., Ahn, J. and Kwak, H. S. (1999). Cholesterol removal from homogenized milk with β-cyclodextrin. *Journal of Dairy Science, 82*(11), 2327-2330.

Macdonald, I. A., Bokkenheuser, V. D., Winter, J., McLernon, A. M and Mosbach, E. H. (1983). Degradation of steroids in the human gut. *Journal of Lipid Research, 24*(6), 675-700.

Mathur, B., Hashizume, K., Musumi, S., Nakazawa, Y and Watanabe, T. (1986). Traditional cheese "Paneer" in India and soy bean food "tofu" in Japan. *Japanese Journal of Dairy and Food Science*, *35*(4), 137-141.

Nguyen, A. T. L., Boakye, P. G., Besong, S. S., Tomasula, P. M. Alamu-Lumor, E. S. (2021). Improvement of physicochemical properties of reduced-cholesterol butter by the addition of β-sitosteryl oleate. *Journal of Food Science, 86*(2), 404-410.

Parmar, Y., Sharma, V., Arora, S., Rani, A and Hazra, T. (2016). Standardization of enzymatic diagnostic kit based method for cholesterol estimation in butter, kalakand, khoa and paneer. *Indian Journal of Dairy Science*, *69*, 443-440.

Schlimme, E. and Kiel, D. (1990). Removal of cholesterol from milk fat. *European Dairy Magazine, 4*, 12-21.

Sharma, R., Nath, B. S and Lal, D. (1999). Approaches for cholesterol removal from milk fat: An overview. *Indian Journal of Dairy and Biosciences, 10*, 138-146.

Tylkin, X. B., Grishina, V. E. and Suidovskii, A. (1975). Photometric investigation of the contents of fat soluble vitamins and cholesterol in dairy products. *Tovarovedenie, 8*, 42.

How to cite this article: Reji A.J., Divya M.P., Rajakumar S.N., Rejeesh R. and A.K. Beena (2024). Manufacture and characterization of Low Cholesterol Paneer Prepared from Buffalo Milk. *Biological Forum – An International Journal, 16*(1): 161-165.