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TOTAL PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF MOSSES FROM YENICE FOREST (IDA MOUNTAIN)

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ABSTRACT:

Recent pharmacological investigations of bryophytes have proven that the active principles present in these plants are quite unique and having potential chemical application and antioxidant capacity. In this study, the volatile components in extracts from Thuidium tamariscinum (Hedw.) Schimp. and Platyhypnidium riparioides (Hedw.) Dixon, Kazdağları (Kalkim-Yenice, Çanakkale, Turkey) were isolated by solid phase micro extraction technique and identified by mass selective detector gas chromatography (GC-MS). Antioxidant capacities of these species were determined by CERAC and CUPRAC methods and phenolic contents by Folin-Ciocalteu method.

Keywords: *Thuidium tamariscinum and Platyhypnidium riparioides; mosses; antioxidant activity; GC-MS; TEAC, CUPRAC methods.*

1. INTRODUCTION

Plant phenolic are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Löliger, 1991). Most phytochemicals in natural agricultural sources have been generally recognized as bioactive or health-promoting compounds, which play an important role in

preventing cardiovascular diseases, cancers, obesity and diabetes, lowering blood cholesterol level, and reducing inflammatory action (Halliwell, 1996). Mosses and liverworts are small, low-growing plants and constitute the phylum Bryophyta, which is phylogenetically placed between vascular plants and algae. Bryophyta has more than 22.000 members all over the world, and nearly 3000 bryophytes are reported to have medicinal value and therefore, the members of this unique division in the plant kingdom are now increasingly used as new sources of pharmaceuticals. One interesting class of bryophytes, the liverworts are being therapeutically used worldwide, especially in Indian and Chinese cultures for the treatment of hepatitis and skin disorders due to their antibiotic, anti- inflammatory, and diuretic properties (Friederich, 1999; Gökbulut et al., 2012; Saroya, 2011). Bryophytes are considered as a "remarkable reservoir" of new, natural products or secondary compounds, many of which have shown interesting biologic activity. These activities can be presented as: antimicrobial, antifungal, cytotoxic, antitumor, vasopressin (VP) antagonist, cardiotonic, allergy causing, irritancy and tumor effecting, insect anti-feedant, insecticidal, molluscicidal, piscicidal, plant growth regulatory, superoxide anion radical release inhibition and 5-lipoxygenase, calmodulin, hyaluronidase, and cyclooxygenase inhibition features of bryophytes (Asakawa et al., 2013). Bryophytes are useful plants as sources of natural products since they grow everywhere in the world. The secondary metabolites identified from mosses belong to terpenoids, flavonoids and bibenzyls, but they are also rich in other compounds such as fatty acids, acetophenols and antimicrobial activity is related to the specific chemical composition, structural configuration of compounds, functional groups, as well as potential synergistic or antagonistic interactions between compounds.

Antioxidant capacity of the moss was found to be higher than certain common plants. High level of antioxidants present in liverworts and mosses can serve as a future source for medicinal and cosmetic purpose. Traditional medicinal use of bryophytes includes different ailments viz. inflammation, skin disease, wound healing (Singh et al., 2006), viral diseases (Frahm, 2004) etc. Bryophytes, especially liverworts, often have distinct odors, suggesting aromatic compounds such as phenols. However, few bryophytes have been linked to actual curative properties and identifiable associated compounds many antibiotics have been isolated from bryophytes, but few have been developed for medical use, despite their demonstrated effectiveness. In Germany, *Ceratodon purpureus* and *Bryum argenteum* are used to cure fungal infections of horses. Several medical uses seem promising, such as anti-leukemic properties and anticancer agents (Glime, 2013). In India, people of Kumaon Himalaya use *Marchantia polymorpha* and *M. palmata* to cure burns, abscesses and to reduce pus formation, while paste of Riccia spp. is applied on the ring worm disease of skin (Pant and Tewari, 1989; Kumar et al., 2000; Dhondiyal et al., 2103).The Gaddi tribes of Himachal Pradesh, India use *Plagiochasma appendiculatum* for the cure of burns, boils and blisters of skin (Kumar et al., 2000; Dhondiyal et al., 2103).The folklore use of bryophytes could be due to certain active compounds having antioxidant capacity. Ethno medicinal use of different bryophytes should be scientifically investigated for active principles in order to bridge between traditional knowledge and pharmacology (Yayintas et al, 2017).

The aim of this study was to investigate the antioxidant activity and volatile component of *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon.

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The present assay was designed to establish the volatile compounds of these mosses and their total antioxidant capacity (TAC), total phenolic content (TPC) using a combination of CERAC, CUPRAC, Folin-Ciocalteu methods.

Extraction of mosess

Water extraction: The dried mosses sample (0.25 g powdered of *Thuidium tamariscinum* or 0.50 g *Plathypnidium riparioides*) was pounded into small parts with a porcelain mortar. The dried powdered aerial parts of the plants were extracted with 50 mL boiling distilled water and were mixed with sonicate for 10 min and orbital shaker for 10 min at 450 rpm. The extract was filtered through a Whatman filter paper into a 50 mL-flask, and diluted to the mark with distilled water. The aqueous extract of bryophytes was prepared just before the experiments so as to prevent any undesired degradation reactions.

Methanol extraction: 0.25 g powdered of *Thuidium tamariscinum* and 0.50 g *Plathypnidium riparioides* mosess were used. These were extracted in stoppered flasks using 100 % (v/v) methanol. Two successive batch extractions were carried out using the stirrer. The first extraction was made with 30 mL 100 % MeOH for 60 min, the second with 20 mL 100 % MeOH for 60 min at 450 rpm. The two extracts were filtered and combined in a single graduated flask and diluted to 50 mL with 100 % MeOH at room temperature.

Gas chromatography-mass spectrometry (GC/MS)

Volatile compounds from moss samples were isolated by solid-phase microextraction (SPME) technique (Pawliszyn, 2012) and identified by Gas chromatography mass spectrometry. 0.2 g of moss sample was weighed in a 40-mL amber-coloured screw-top vial with a hole-cap polytetrafluoroethylene/silicon septum (Supelco, Bellefonte, USA), and 0.2 g of NaCl and 5 mL of distilled water were added to the vial. The vial was kept at 40 °C in a water bath for 20 min to equilibrate volatiles in the headspace. Then, an SPME (2 cm to 50/30 µm DVB/Carboxen/PDMS, Supelco, Bellefonte) needle was inserted into the vial. The SPME fibre was exposed at a depth of 2 cm in the headspace of the vial. Then, the SPME needle was immediately injected into a GCO or GC–MS column.

Volatiles were tentatively identified by GC–MS. A nonpolar HP5 column (30 m × 0.25 mm i.d. × 0.25-µm film thickness; J&W Scientific) was used for separation of volatiles. The GC–MS system consisted of an HP 6890 GC and 7895C mass-selective detector (MSD; Agilent Technologies, Wilmington, DE, USA). The GC oven temperature was programmed from 40 to 230 °C at a rate of 10 °C·min^{-1}, with the initial and final hold times of 5 and 20 min, respectively. Helium was used as a carrier gas at a flow rate of 1.2 mL·min^{-1}.The MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 35 to 350 amu; scan rate, 4.45 scans·s^{-1}. The identification of flavor compounds was based on comparison of the mass spectra of unknown compounds with those in the databases of the National Institute of Standards and Technology and Wiley Registry of Mass Spectral Data. Flavor compounds were quantified based on their relative abundances. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany)

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CUPRAC spectrophotometric assay of total antioxidant capacity

1mL 10 mM cupric chloride, 1 mL 7.5mM neucuproine, 1 mL 1 M ammonium acetate buffer (pH:7) and 1 mL water were mixed. 0.1 mL bryophytes extracts were added in this mixture. The samples were incubated for half an hour at room temperature, absorbance against a reagent blank was measure at 450 nm. The results were expressed as mmol catechin per gram dry mosses (Apak et al.,2004).

CERAC spectrophotometric assay of total antioxidant capacity

1 mL of 2.0 x 10^{-3} M Ce(IV) solution + x mL of the bryophytes extract was placed into a test tube and diluted to 10 mL with H_2O. The mixture was allowed to stand for 30 min at room temperature and the 320-nm absorbance (A_{320} nm) was measured. The total antioxidant capacity was determined and results expressed as mmol equivalents dry weight (Ozyurt et al., 2007). The molar absorptivity of catechin in the CERAC method is $\varepsilon = 1.31\times104$ L mol^{-1} cm^{-1}. The results were expressed as mmol catechin per gram dry mosses (Ozyurt *et al*., 2007). The molar absorptivity of catechin in the CERAC method is $\varepsilon = 1.31\times104$ L mol^{-1} cm^{-1}. The results were expressed as mmol catechin per gram dry mosses.

Folin-Ciocalteu assay of total phenolic content

Samples were analyzed spectrophotometrically for contents of total phenolic by a *Folin–Ciocalteu* colorimetric method (Singleton et al., 1999). Volume of 1.5 mL of deionized water and 0.5 mL of the extract were added to a test tube, followed by addition of 2.5 mL of Lowry C solution was added, and the mixture was allowed to stand for 10 min. At the end of this period, 0.25 mL of Folin reagent was added, and 30 min were allowed for stabilization of the blue color formed. The molar absorptivity of gallic acid in the $AlCl_3$/ $NaNO_2$ colorimetric method is $\varepsilon = 5.14\times10^3$ L mol^{-1} cm^{-1}. The results were expressed as mmol gallic acid per gram dry mosses.

2. RESULTS AND DISCUSSION

GC-MS identification of water extracts

The mosses were sampled from Yenice forest from Ida Mountain (Kazdağ). They are abundant in nature. Data obtained from GC-MS from the sample of water extracts of *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon were given in Figure 1,2 and Table 1 respectively.

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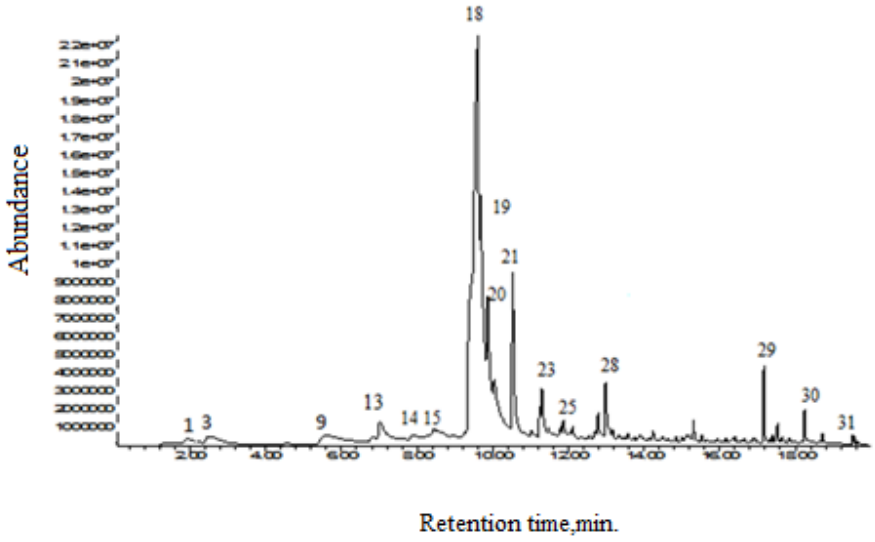

Figure 1. Mass spectrometry-gas chromatogram of the components of *Thuidium tamariscinum*; Number refer to the compounds given in Table 1.

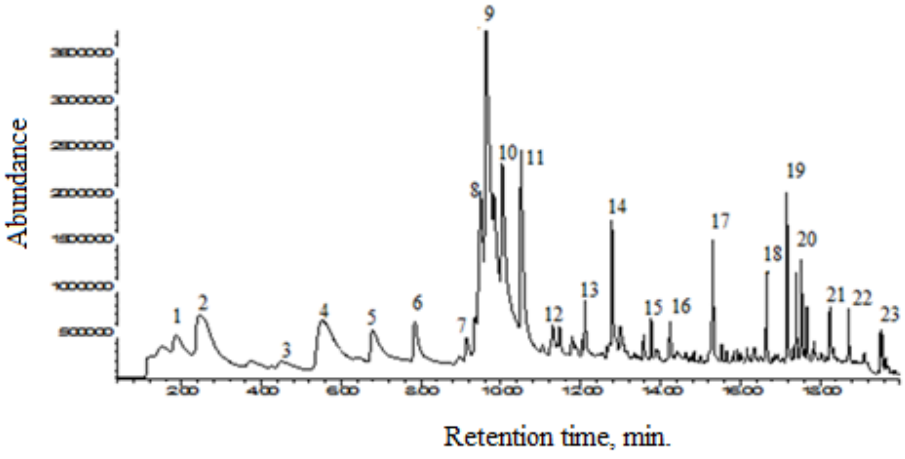

Figure 2. Mass spectrometry-gas chromatogram of the components of *Plathypnidium riparioides*; Number refer to the compounds given in Table 1.

A large number of compounds were determined from water extract of *Thuidium tamariscinum* and *Platyhypnidium riparioides* by GC-MS. Among these compounds with higher abundance were given in Table 1.

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Table 1. GC-MS analysis of *Thuidium tamariscinum* and *Plathypnidium riparioides* (water extract) component.

	Compound No	Retention time (min)	Component	Properties	Abundance %
Thuidium tamariscinum					
	1	1.93	Chloroform	plant volatile	1.00
	2	2.49	Trimethylsilyl trifluoroacetate		0.65
	3	2.63	Dimethyl-silanediol		1.36
	4	3.79	1-Pentanol	odor	0.35
	5	4.37	Benzenemethanol, .alpha.-(1-aminoethyl		0.09
	6	4.40	3-Methoxyamphetamine	stimulant drug	0.01
	7	4.54	Hexanal	used in the flavor industry to produce fruity flavors	0.48
	8	5.07	4-(2-Methylamino)ethyl)pyridine	nucleophilic catalyst	0.05
	9	5.16	2,3-Dimethylamphetamine	stimulant drug	0.10
	11	6.41	N-ethyl-1,3-dithioisoindoline	psychoactive drug	0.26
	12	6.82	Hexamethyl cyclotrisiloxane	polymeric, oil	0.61
	13	7.02	1,3-cis,5-cis-octatriene	herbal scent, plant defense and anti-fungal properties	2.73
	14	7.90	2-Amino-5-methylbenzoic acid		1.13
	15	8.44	2,4,6-Octatriene, all-E-		2.33
	16	8.67	1,2-dimethyl-1,4-cyclohexadiene	terpenoids	0.92
	17	8.95	1,2-Dimethyl-1,4-cyclohexadiene	mushroom alcohol	0.91
	18	9.60	1-Octen-3-ol	sharp, sweet odor reminiscent of butterscotch and acetone.	27.30
	19	9.69	Neopentyl ethyl ketone	organosilicon	5.59
	20	10.04	Octamethyl-cyclotetrasiloxane	fruit-like taste.	5.59
	21	10.53	2-Butenedioic acid (E)-, bis(2-	fatty oily sweet	5.65

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			ethylhexyl) ester	fruity	
	22	11.24	2-Octen-1-ol, (E)-	Food flavor/aroma component	0.65
	23	11.30	Bromoacetic acid, decyl ester	fruity, pungent odor	1.92
	24	11.48	Decamethyl-tetrasiloxane	slightly volatile	0.83
	25	11.80	Trifluoroacetic acid, 4-methylpentyl ester	sharp odor similar to vinegar	0.60
	26	11.87	Nonanal	A colorless, oily liquid, nonanal is a component of perfumes	0.90
	27	12.79	p-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate	acrid odor	1.12
	28	13.16	1-Dimethylthexylsilyloxybutane		0.57
	29	17.53	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane		0.52
	30	18.24	Ethylparaben	antifungal preservative. food additive	0.61
	31	19.51	Ethanedioic acid, bis(trimethylsilyl) ester		0.18

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			Table 1 continued		
	Compound No	**Retention time (min)** **(daki (min)**	**Component**	**Properties**	**Abundance %**
Plathypnidium riparioides	1	1.87	Chloroform	plant volatile	2.74
	2	2.45	Trimethylsilyl trifluoroacetate		6.49
	3	4.50	Hexanal		1.70
	4	5.54	2,3-Dimethylamphetamine		5.76
	5	6.78	Hexamethyl cyclotrisiloxane	polymeric, oil	2.91
	6	7.84	N-ethyl-1,3-dithioisoindoline		2.96
	7	8.97	1,2-Dimethyl-1,4-cyclohexadiene		0.70
	8	9.50	2,6,10,14-tetramethyl-pentadecane		3.75
	9	9.63	1-Octen-3-ol		8.29
	10	10.04	Octamethyl-cyclotetrasiloxane	organosilicon	7.56
	11	10.51	2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester		5.73
	12	11.30	Bromoacetic acid, decyl ester		1.2
	13	12.11	2,6,10,14-tetramethyl- Pentadecane		1.30
	14	12.99	p-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate		1.13
	15	13.99	2-Ethylacridine		0.36
	16	14.14	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-		0.21
	17	15.31	Quinoline, 4-(4-chlorophenoxy)-8-fluoro-2-trifluoromethyl-		1.81
	18	16.93	Geosmin		0.26
	19	17.18	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-		1.47
	20	17.53	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane		0.93
	21	18.53	Quinoline, 4-(4-chlorophenoxy)-8-fluoro-2-trifluoromethyl-		0.16
	22	18.71	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester		0.67
	23	19.71	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl		0.15

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Determination of total antioxidant capacity and phenolic content

The total antioxidant capacity of mosses from methanol extracts and aqueous infusion determined by CERAC and CUPRAC methods. The results of the analysis calculated as mmol catechin/g dry mosses and given in Table 2 and Table 3. The phenolic content of mosses from methanol extracts and aqueous infusion determined by Folin- Ciocalteu method. The results of the analysis calculated as results are calculated as mmol gallic acid/g sample and given in Table 2 and Table3.

Table 2. The total amount of antioxidant capacity and phenolic content of mosses with 100 % (v/v) methanol

Samples	**CERAC (mmol catechin/ g mosses)**	**CUPRAC (mmol catechin / g mosess)**	**FOLIN- CIOCALTEU (mmol gallic asit/ g mosses)**
Thuidium tamariscinum	0.01200	0.00701	0.12900
Plathypnidium riparioides	0.00602	0.00327	0.06290

Table 3. The total amount of antioxidant capacity and phenolic content of aqueous infusion of mosses.

Samples	**CERAC (mmol catechin/ g mosses)**	**CUPRAC (mmol catechin / g mosess)**	**FOLIN- CIOCALTEU (mmol gallic asit/ g mosses)**
Thuidium tamariscinum	0.00403	0.00018	0.00632
Plathypnidium riparioides	0.00210	0.00039	0.00461

3. CONCLUSION

This study presents the results of a moss extracts of *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon were collected from Yenice forest to Ida Mountain (Kazdagı) Canakkale, Turkey, by chemical composition antioxidant activities.

These results are the proof that *Thuidium tamariscinum* (Hedw.) Schimp. and *Platyhypnidium riparioides* (Hedw.) Dixon extract possesses potent antioxidant activity. These significant proportions of the antioxidant activity are caused by phenolic substances in mosses structure. To sum up, here it was demonstrated that the extract of the mosses investigated here have great potential to be used in medicine, cosmetics and pharmaceutical applications as well as food and agricultural use.

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THE DETERMINATION OF NH_4^+ CATION AND Al METAL CONCENTRATIONS IN WATER OF SWIMMING POOLS IN CENTER OF CANAKKALE, TURKEY

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ABSTRACT

In current study, NH_4^+ cation and Al metal concentrations in summer period were determined by ultraviolet-visible (UV-VIS) spectrophotometric technique in the total 6 swimming pools found in the province of Canakkale (Turkey). Merck Kits equivalent to EPA, APHA, ISO and DIN standards were used in spectrometric analyses. The monthly quality parameter results in this study were evaluated according to various limit standard values of different countries. As a result, NH_4^+ cation and Al metal concentrations varied between 0.003 and 0.999 mg L^{-1} (0.073±0.139 mg L^{-1}), 0 and 0.652 mg L^{-1} (0.141±0.068 mg L^{-1}) respectively.

Keywords: *Canakkale, Swimming pool waters, ammonium cation, Al metal*

1. INTRODUCTION

Swimming is one of the most popular aquatic activities in industrialized countries. During busy periods, the quality of pool water may be compromised. Indeed, swimmers bring microorganisms and organic substances (saliva, sweat, cosmetics, sunscreen and urine) with them into the water, which strongly contributes to water contamination (Sakkas *et al.,* 2003; Kanan and Karenfil, 2011; Keuten *et al.*, 2012; Uysal *et al*., 2017).

Ammonia in water is an indicator of bacterial, fecal and animal wastes is being mixed into the water (Anonymous, 2006; Uysal *et al*., 2017). Pool water resources NH_4^+, the filling water, human origin (fecal and urine contamination) and is caused by biofilm formation

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(Güllüoglu, 2010; Uysal *et al*., 2017). Ammonium is just quickly oxidized to nitrite in oxygen environment and then transformed into nitrate. Nitrification is carried out by bacteria such as *Nitrosomonas* and *Nitrobacter* group bacteria (Mutluay and Demirak, 1996; Uysal *et al*., 2017).

Organic substances which cause water discoloration are usually removed from water by coagulation (clotting), precipitation and filtration (Yaşa, 1999; Uysal *et al*., 2017). Before the filtration, the stability of small particles (electrical) is deteriorated thanks to the addition of the flock material into pool water and hence agglomeration of particles is provided. For flocking, chemical (e.g. addition of colloidal substances such as $Al_2(SO_4)_3$ to water) is used (Bölükbasioglu, 1993; Uysal *et al*., 2017). The flocculation decrease chlorine consumption in the pool and hence eye diseases and BK formation which cause bad smell are avoided (Akkaya, 1993; Uysal *et al*., 2017). Aluminium (Al) is known to be non-toxic element. However, excessive amounts of intake have been shown to influence the nervous system and cause anemia. Al is an element also used in Alzheimer and diabetes diseases (Canadab, 2003; Uysal *et al*., 2017).

Limit concentration values of the Turkish Health Ministry for NH_4^+ cation and Al metal are 0.50 and 0.20 mg L^{-1}, respectively (Anonymous, 2011; Uysal *et al*., 2017). In order to evaluate some quality standard levels of swimming pools found in the province of Canakkale (Turkey), this study on determination of NH_4^+ cation and Al metal concentrations in water of swimming pools in center of Canakkale has been conducted in summer period (July, June and August, 2013) due to the intensive tourism activity in this period..

2. MATERIALS AND METHODS

2.1. Study area and period

In this study, 6 swimming pools as a study area (Figure 1) were selected. Pool water samples were monthly collected from sampling locations (swimming pools) in summer period of 2013 (June, July and August, 2013) in Canakkale province (Figure 1). The sample numbers Çanakkale, 33-36 (Figure 1).

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Figure 1. Water sampling points for swimming pool in the summer period in the provinces of Canakkale, Turkey (Sample numbers : Çanakkale, 33-36), (Uysal *et al.,* 2017).

Pool water samples were collected from special points where the water flow is the lowest or swimmers are the most intense. Moreover, special sampling depths and points were just under surface (0.20 m) and about one meter away from the edge line of the pools. The collected samples were put in clean polythene sample bottles (1.00 L) and stored in deep freezer at -21.0 ^{o}C until analyses. In this study, samples from the total 6 swimming pools were analyzed for each parameter..

2.2. Analytical methods

In current study, NH4+ cation and Al metal concentrations in summer period were determined by ultraviolet-visible (UV-VIS) spectrophotometric technique in the total 6 swimming pools found in the province of Canakkale (Turkey). Thermo Aquamate Brand

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Spectrophotometer was used for analyses. For analyses, Merck Kits equivalent to EPA, APHA, ISO and DIN standards were used.

Ammonium nitrogen (NH4+-N) occurs partly in the form of ammonium ions and partly as ammonia (Uysal et al., 2017). A pH-dependent equilibrium exists between the two forms. In strongly alkaline solution ammonium nitrogen is present almost entirely as ammonia, which reacts with hypochlorite ions to form monochloramine (. This, in turn, reacts with a substituted phenol to form a blue indophenol derivative that is determined photometrically. The method is analogous to EPA 350.1, APHA 4500-NH3 F, ISO 7150-1, and DIN 38406-5. Analysis measuring range is ranged from 0.01 to 2.00 mg L-1 NH4+ -N and 0.01 to 2.58 mg L-1 NH4+. In the production control, the following data were determined in accordance with ISO 8466-1 and DIN 38402 A51: Standard deviation of the method ± 0.0146 (mg L-1 NH4+-N), coefficient of variation of the method ± 1.40 (%), confidence interval ± 0.035 (mg L-1 NH4+-N), Sensitivity: Absorbance 0.010 A corresponds to 0.009 (mg L-1 NH4+-N), accuracy of a measurement value max ± 0.052 (mg L-1 NH4+-N) (Merck 2014).

In weakly acidic, acetate-buffered solution aluminium ions react with chromazurol S to form a blue-violet compound that is determined photometrically (Uysal et al., 2017). The method is analogous to APHA 3500-Al-B and DIN ISO 10566 E30. Analysis measuring range is 0.02 to 2.00 mg L-1 Al unless otherwise stated. In the production control, the following data were determined in accordance with ISO 8466-1 and DIN 38402 A51 (10-mm cell): Standard deviation of the method ± 0.012 (mg L-1 Al), coefficient of variation of the method ± 1.70 (%), confidence interval ± 0.03 (mg L-1 Al), Sensitivity: Absorbance 0.010 A corresponds to 0.001 (mg L-1 Al), accuracy of a measurement value max. ± 0.008 (mg L-1 Al) (Merck, 2014).

3. RESULTS AND DISCUSSION

The found concentration of NH_4^+ cation and Al metal in water of swimming pools water in center of Canakkale, are giving in Table 1 and Figure 2,3.

Average summer results showed that NH_4^+, concentrations varied between 0.003 and 0.999 mg L^{-1} (0.073±0.139 mgL^{-1}) . Based on both maximum and average values, NH_4^+ concentration increased from June to August probably due to the increasing touristic population.

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Table 1. Descriptive statistical results of NH_4^+ concentrations in swimming pools in the summer period in the provinces of Canakkale, Turkey (*n*: Number of observation; *l* : The number of samples exceeding the limit concentration values specified by Turkish Health Ministry).

Month 2013	Parameters (mg L^{-1})	*n*	Maximum Values	Minimum Values	Average Values	Standard Deviation	Analysis Accuracy	Limit Values	*l*
June	NH_4^+	44	0.4425	0.0102	0.0460	0.0658	±0.059	0.50	0
July	NH_4^+	44	0.9639	0.0032	0.0756	0.1541	±0.059	0.50	1
August	NH_4^+	44	0.9992	0.0123	0.0967	0.1983	±0.059	0.50	3
Summer Average	NH_4^+	44	0.9992	0.0032	0.0728	0.1394	±0.059	0.50	4

Figure 2 showed that NH_4^+ concentrations varied between 0.0102 and 0.9992 mg L^{-1} during the sampling period.

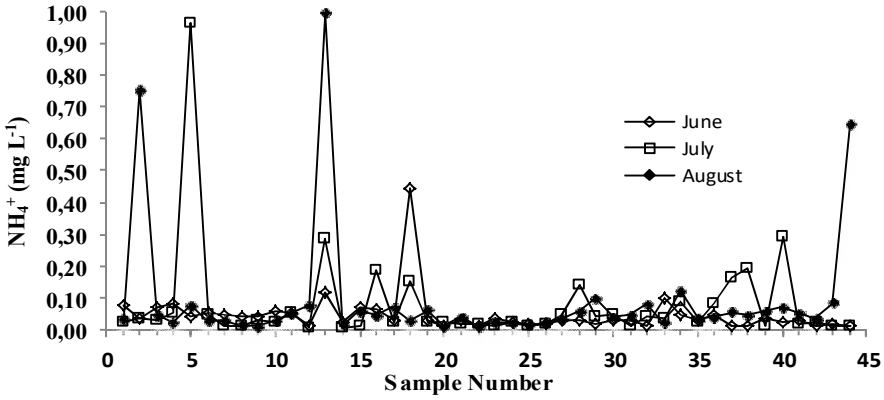

Figure 2. The concentration of NH_4^+ in swimming pool waters in the summer period in the provinces of Canakkale, Turkey (Sample numbers: Çanakkale, 33-36).

Al metal variation

Al metal variations in swimming pool waters in the summer period in the province of Canakkale, Turkey are giving in Table 2 and Figure 3.

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Table 2. Descriptive statistical results of Al concentrations in swimming pools in the summer period in the provinces of Canakkale, Turkey (*n*: Number of observation; *l* : The number of samples exceeding the limit concentration values specified by Turkish Health Ministry).

Month 2013	Parameters (mg L^{-1})	*n*	Maximum Values	Minimum Values	Average Values	Standard Deviation	Analysis Accuracy	Limit Values	*l*
June	Al	44	0.3596	0	0.0325	0.0529	±0.008	0.20	1
July	Al	44	0.6522	0	0.0500	0.0956	±0.008	0.20	1
August	Al	44	0.3736	0	0.3390	0.0543	±0.008	0.20	1
Summer Average	Al	44	0.6522	0	0.1405	0.0676	±0.008	0.20	3

Average summer results showed that Al concentrations varied between 0 and 0.652 mg L^{-1} (0.141±0.068 mg L^{-1}) . Although average Al concentration increased from June to August probably due to the increasing swimmer population based on both average values, maximum values of Al (0.6522 mg L^{-1}) was July and August, respectively (Table 2). However, although the all maximum Al values were over the limit values for pool water standards of Turkish Health Ministry and the standards of some European countries, the all average values were considerably under all these limit values (Table 2).

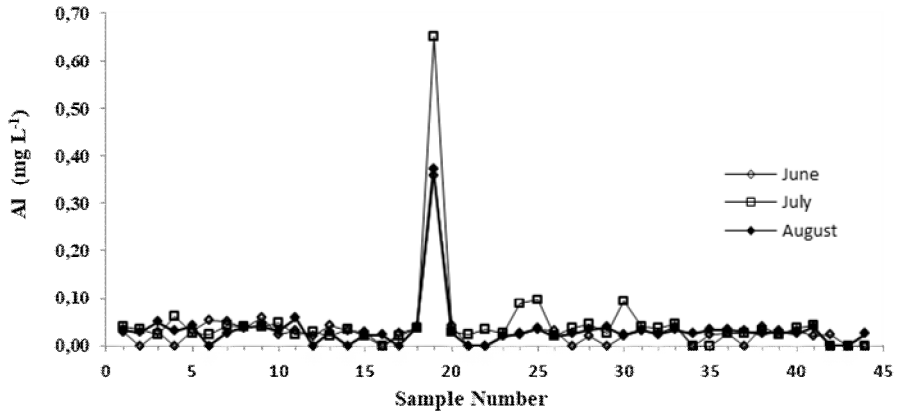

Figure 3. Variations of Al in swimming pool waters in the summer period in the provinces of Canakkale, Turkey (Sample numbers: Canakkale, 33-36).

Results revealed that Al concentrations varied between 0 and 0.6522 mg L^{-1} during the sampling period (Figure 3). Unhealthy pool water ratio for Al was calculated as 2.27%.

4. CONCLUSIONS

The results of NH_4^+ indicated that there were suitable aerobic nitrification conditions due to the high dissolved oxygen concentrations in the pool waters. It is known that NH_4^+ very quickly converts to NO_3^- due to the nitrification in aerobic conditions (Mutluay and Demirak, 1996; Putz, 2008, Uysal *et al.,* 2017) and partly NH_4^+ were under standard limit values (Table

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1). In a study on microbiological and chemical qualities of 144 swimming pool waters (69 indoor, 75 outdoor) for three-year period (2010-2012) in Bologna (Italy), Dallolio *et al.* (2013) using same methodology (spectrometric method) in this study revealed that noncompliance rate to standard limit value (unhealthy pool water ratio) were 1.20 and 0.00% in the indoor swimming pool waters and outdoor pool waters, respectively (Uysal *et al.,* 2017).

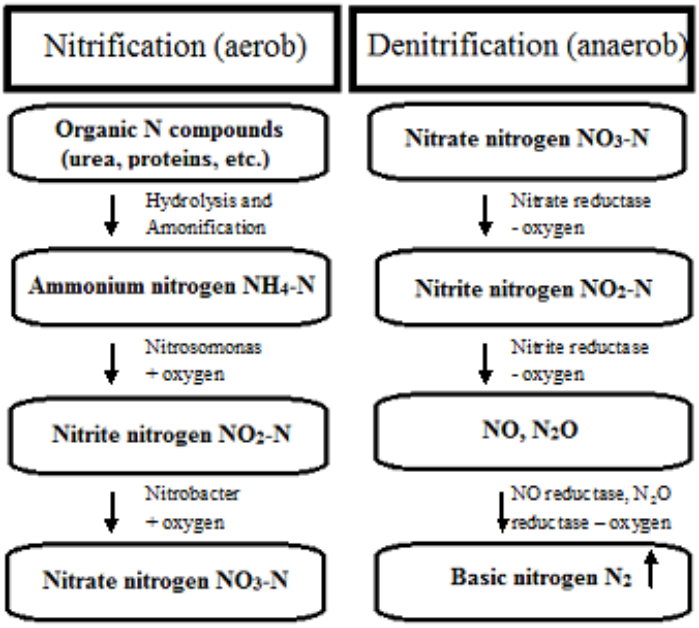

Figure 4. Nitrification and denitrification (Pütz, 2008; Uysal *et al.,* 2017).

On the other hand, non-human factors such as birds and plants. It is known that faces of the birds contain higher nutrient values than faces of the human (Tiffany *et al.,* 2015; Uysal *et al.,* 2017). For solution of such problems, it is important to get sanctions and obligations for hygiene controls and periodic changes of pool waters in legal manner (Uysal *et al.,* 2017).

As a result, due to the fact that Canakkale province has important tourism activity in Turkey, pool waters in such tourism regions should be monitor in view of not only chemical (NH_4^+ cation and Al metal) and physical quality parameters (Temperature, pH and DO), but also microbiological (*Salmonella* and *Vibrio* etc.) quality parameters (Uysal *et al.,* 2017).

Conflict of Interest Statement

The authors declare no conflict of financial, academic, commercial, political, or personal interests.

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HISTOPATHOLOGICAL RESEARCH AND DIAGNOSIS OF IMPORTANT PAINTING METHODS

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ABSTRACT

Both diseases can safely in experimental research need to the histopathological diagnosis many coloring method utilized. This application of Immunohistochemistry and İmmunfloresan coloring methods stands out in terms of definitive diagnosis. Our country and other countries in which research scientists or Diagnostics these methods and which use antibodies to investigate the research planned and many Assembly-style research. İmmunfloresan coloring method, especially of some diseases affecting the immune system, resulting in the tissues of immunoglobulin and complement in the display of the Immunohistochemical staining method used to diagnose cancerous tissues. Both the painting cut early diagnosis of cancer in particular methods for specific investigations and used as safe.

***Key Words**: Immunohistochemistry, Immunoflourescence, Histology, Pathology, Histopathology*

1. INTRODUCTION

In the case of experimental investigations, histopathologic changes in the cells are revealed by using immunohistochemistry and immunofluorescence staining methods by making use of many different staining kits. Immunohistochemical staining is a differential diagnosis method that takes advantage of immunology based on identification and demonstration of building blocks or chemical reactions in cells with enzymes marked with a special color. Identifies the type of tumor, the tissue from which it is obtained, the normal or extraordinary products it produces, and thus determines the tumor type or whether the drugs used in the tumor-specific treatment can be applied. Immunofluorescence staining etiology is based on the principle of recognizing certain skin, kidney, vascular diseases with immunologically mediated mechanisms, revealing the stored immunocomplexes with fluorescence marked dyes and evaluating the existing fluorescence with dark field specific light filters.

In our study, it was aimed to reveal the importance of dyeing methods and dyeing kits in the field of histopathology with the literature information.

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2. METHOD

Dyeing kits and dyeing methods, which have an important role in revealing the histopathological changes in cells and tissues, have been investigated by using the literature information.

3. RESULTS

In the literature survey, many immunohistochemical and immunofluorescent staining methods have been used to reveal histopathologic changes in tissues and cells.

Elagoz et al. (2006) reported that an immunohistochemical panel consisting of CD117, CD34, actin, desmin should be applied to distinguish the other mesenchymal tumors from gastric intestinal stromal tumors of mesenchymal tumors.

Du et al. (2008) showed that CD44 was a robust marker for the diagnosis of colorectal cancer.

Niflioğlu and Nebe (2014) used Immunoglobulin G antibody and Complex 3 (C3) antibodies by Direct Immunofluorescence to detect Immunoglobulin G and Complex 3 accumulation in subcutaneous basement membrane in Gestational pemphigoid (GP) disease with autoimmune subepidermal bullous dermatosis.

Ma et al. (2013) identified IgG and C3 localization on the basal membrane epinephrine side using IgG and C3 antibodies by immunofluorescence method in the diagnosis of membranous glomerulonephritis, the most common cause of nephrotic syndrome in adults.

Ozsan et al. (2013) evaluated estrogen receptor and progesterone receptor immunoreactivity and percentages of P53, c-erb B2, and Ki67 immunoreactivity staining to assess receptor status in breast cancer, the most common malignancy in women at the beginning of cancer-related death causes in women today.

Liu et al. (2016) used CK5 / 6, CK14, E-cad, CD24, CD44, CLDN3, CLDN4, CLDN7, Vimentin, AR and EGFR antibodies to differentiate subtypes of breast cancers.

Coskuner et al. (2000) investigated PSA immunoreactivity in prostate cancer. Investigators have shown that immunohistochemical staining of prostate tissue with PSA reveals the prostatic origin of the tissue with a few exceptions, especially with poorly differentiated tumors spreading to the prostate and bladder; Stated that the presentation of prostatic origin in metastatic cancers is valid for the detection of large pelvic masses that go through multiple organ involvement.

Prasad (2005) stated that immunohistochemical staining of thyroid nodules is supportive in the diagnosis of thyroid tumors, using Galectin-3, Fibronectin-1, Cited-1, Hbme1 and Cytokeratin-19 immunohistochemical staining kits to increase the correct diagnosis rate.

Demirhan et al. (2011) positively detected cytokeratin, vimentin, CD34 immunoreactivity by immunohistochemical staining of the pleura's solitary fibrous tumor and emphasized its importance in terms of differential diagnosis.

Turk et al. (2014) reported that the differential or differential diagnosis of angiomyomas from multiple reactive and neoplastic diseases, which may exhibit myxoid

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degeneration, is very important, and that the positivity or negativity of vimentin, S100, desmin and smooth muscle actin immunoreactivity is the most important.

Diagnosis of Kaposi's sarcoma, which is a type of tumor caused by Adisa et al. (2013), Human herpes virus 8 (HHV8) or Kaposi's sarcoma-associated herpes virus (KSHV), and after widespread appearance in AIDS patients in the 1980s, Vimentin and smooth Muscle actin (SMA) antibodies are the most reactive.

Tyagi et al. (2002) reported that Bcl2 expression in central nervous system tumors increased 2-6 fold compared to normal tissue.

Muratlı et al. (2013) investigated the iNOS immunoreactivity in the gastric mucosa by giving mussels to the rats and found that the mussel-fed rats removed from the heavy metal-containing regions were iNOS-positive staining in the gastric mucosa. Gezen et al. (2013) investigated iNOS immunoreactivity in ovaries of mussel-fed rats and found iNOS positive staining in ovaries of mussel-fed rats extracted from heavy metal-containing regions.

4. DISCUSSION

When literature information is evaluated, Liu et al. (2016), CK5 / 6, CK14, E-cad, CD24, CD44, CLDN3, CLDN4, CLDN7, Vimentin, AR and EGFR antibodies. (2013) estrogen receptor and progesterone receptor, P53, c-erb B2 and Ki67 antibodies, Hammond et al. (2010) used estrogen receptor and progesterone receptor.

Du et al. (2008) used CD44 antibody in colorectal cancer, Akagi et al. (2000) reported that the Vascular endothelial growth factor-C antibody in the regional lymph nodes with the colon, Szajewski et al. (2015) used the VEGF-C antibody. George et al. (2001) evaluated the association of the angiogenic cytokines VEGF-A, VEGF-C and VEGF-D receptors VEGFR-2 and VEGFR-3 in metastatic spread of colorectal cancer (CRC).

Muratlı et al. (2013) and Gezen et al (2013) investigated iNOS immunoreactivity in rat stomach and liver by immunohistochemical staining method.

5. RESULTS

Immunohistochemistry and immunofluorescence staining methods have been used frequently with specific antibody kits to determine histopathologic changes in tissues and cells.

6. RECOMMENDATIONS

It is thought that immunohistochemical and immunofluorescent staining methods should be used with appropriate antibody kits in case of academic studies and histopathologic changes in cells and tissues which are important data in disease diagnosis are determined.

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DETERMINATION OF THE QUALITY OF RAW MILK FROM BLACK AND WHITE COWS FROM BIGA (CANAKKALE, TURKEY)

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ABSTRACT

In this research, the subject of inquiry is whether the raw milk in Havdan, Güleçköy and Gürçeşme villages of Biga region is produced according to food safety standards as well as their somatic cell count and chemical characteristics.

For this purpose, 32 samples from Havdan village, 68 from Güleçköy and 16 singular samples from Gürçeşme village. Fat, fatless dry material, protein, somatic cell count, freezing point, cryoscope water amount, lactose and density values of the raw samples have been inquired. Somatic cell count of the produced raw milk turned out to be higher than estimated values in "Turkish Food Codex Raw and Heat Treated Drinking Milk Communiqué Türk Gıda Kodeksi Çiğ ve Isıl İşlem Görmüş İçme Sütleri Tebliği". With precautions to be followed in companies, remarkable outcomes will be accomplished in order to decrease somatic cell count.

Keywords: *Raw milk, quality, somatic cell, chemical characteristics, Biga, Turkey.*

1. INTRODUCTION

One or more cows are breast secretions outside the untreated colostrum (mouth milk) which is obtained by milking a goat, sheep or mandarin, which is not heated above 40 ° C or has an equivalent effect. In milk technology, which will be described in another way, raw milk is milk which is milked at regular intervals and thoroughly from the milk animal's milk, then cooled, no components are taken in, no milk is added to the milk factories to be processed.

Raw milk

Milk should be obtained in conditions of general health which do not show signs of infectious disease which can be passed on to humans, taste of smell, smell and appearance, no visible disease, no abnormal discharge from the back of the animal other than the period of fever, no diarrhea and fever, no intestinal disease or significant breast inflammation.

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Healthy raw milk should be derived from cows and cattle that have not been cured and that are likely to be dangerous or dangerous for human health, from cows that have not come to the end of the lactation period. It should be gathered from cows and cattle's that;

Quality of raw milk

It can be defined as milk that is gathered from a healthy animal under healthy and clean conditions, has distinctive color, taste, structure and composition and none of its components (milk fat) is taken away or no other material (water, soda) added inside (Berberoğlu, 2011).

We can define high quality milk as, milk that is milked from healthy animals under hygienically conditions, is quickly cooled after milking process and kept cool until it is delivered to the factory, has superior sensorial properties, standard chemical composition, untouched biological properties, low bacteria count, low enzyme activity and none of its components is taken away or no new materials added inside.

Quality milk issues

Firstly, there should be no nutrients or preservatives in the milk composition. Also, the total number of bacteria in the raw milk should be very low and should not contain pathogenic bacteria.

Quality criteria of raw milk

Quality criteria in raw milk can be listed as follows;

- ✓ Color and appearance: it must be porcelain white, matt, clean, mildly yellowish.
- ✓ Taste and smell: mildly sweet, fatty, distinctive taste and smell but not an unfamiliar taste or smell.
- ✓ Physical state: Opaque, liquid, a little heavier than water, in a distinctive state that can form cream.
- ✓ Acidity: Desired state is between 6.4 and 6.8 pH values and 6.5 and 7.5°SH titration acidity. Titration acidity going over 8°SH shows a rapid acid increase. If titration acidity goes over 10°SH, clotting occurs during heating. Milk that has titration acidity lower than 5°SH or pH value over 6.8 is considered abnormal.
- ✓ Dirt amount: Milk that contains up to 3 mg dirt per 100 ml milk is considered as `extra class`, up to 6 mg dirt / 100 ml as `first class` and up to 10 mg dirt/ 100 ml as `second class`.
- ✓ Inhibitor: Raw milk shouldn't contain any inhibitors or antibiotics.
- ✓ Composition: It must have the composition value of normal cow milk for the species it belongs to.

Raw milk quality affect

- ✓ Species and breed of the animal,
- ✓ Physiologic state (lactation period, pregnancy)
- ✓ Age of the animal
- ✓ Animal's health
- ✓ States of health of employees

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- ✓ Feeds and feed additives
- ✓ Feeding method
- ✓ Animal feeding
- ✓ Animal welfare
- ✓ Milking management and type
- ✓ Human and Season

2. MATERIAL AND METHOD

In this study, important physical and chemical analyzes were carried out in determining the quality of raw milk. From these analyzes, physical, sensory tests, color, odor, taste, consistency and appearance tests were performed. Milk samples were taken from milk collection centers from different villages of Biga. In dairy collecting areas, milk sampling cups and raw milk prefixes were taken to the laboratory. In addition, analysis of specific weight, freezing point, refractometer index, dry matter, ash content, titration acidity, antibiotic and similar inhibitor control, protein ratio, total bacteria number and somatic cell number were analyzed from chemical analyzes.

3. PHYSICAL ANALYSIS

Sensorial tests

Sensorial tests help us to acquire knowledge about general properties of milk and its production and preservation.

Color

Color of milk is generally white even though it shows some changes depending on species and breed of the animal, individuals and feeding conditions. Apart from its normal color, milk can be a different color depending on physiologic effects, activity of some organisms, bleeding of breasts or water and dyes added in milk.

Smell-taste

Freshly squeezed milk has its own unique taste and odor. Normal taste and smell of milk changes with the effects of many factors. Most significant ones are; feeds and medicine that are given to the animal, diseases, microorganism and enzyme activities in milk, catalytic effects of metals such as copper, iron, etc. interacting with light and oxygen

Viscosity and appearance

Normal milk has a certain viscosity and appearance. Thread-like elongation with high viscosity or pouring fast like water are seen in rigged and spoilt milk. A sandy, pebbly and earthy structure and abnormality can be seen on milk's appearance. These cases are seen in stale and defective milks.

Specific weight

Specific weight of milk, which means weight of 1 ml milk at 15.55°C is averagely 1.032 gr dry matter of milk increases specific weight. Volume increase without change in weight decreases specific weight. Specific weight of newly milked milk is smaller than its specific weight a couple hours after being milked. Reason of this is; decrease in volume of fat globules with time and evaporation of gases.

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Freezing point

It is important because it helps detecting the rig of adding water to the milk and gives accurate information about the amount of water added. Milk freezes at -0.55°C. Boiling the milk decreases the amount of dissolved materials in the composition of milk and therefore increases freezing point. It means; the closer freezing point of milk gets to 0°C, the more the amount of water added in milk.

Refractometer index

Light refraction powers of solutions is constant under certain conditions. Refractometer index of milk changes in a very tight boundary between 1,3440 and 1, 3480.

Chemical

Dry matter

Dry matter is important not only for showing componential wealth of milk, but it is also important because it contains fatless dry matter that helps distinguishing the presence of water that can always be added, due to it being a less volatile substance (Önal et al., 2007).

Ash

Ash determination analysis in milk isn't done often. Studies show that mastitis disease decreases lactose amount and increase NaCl amount in milk, therefore increase the amount of milk ash. Also, milk ash is a low volatile substance and low amount of milk ash shows that water was added and high amount of milk ash shows that inhibitors were added inside the milk.

Fat

Fat rate analysis is one of the most important milk analyses. Fat being constantly variable in the composition of milk, not depending on milk serum much and its rate decreasing by adding water makes fat something to be considered. Therefore fat analysis is one of the analyses that is done in small milk dairies along with milk factories all the time.

Acidity

Newly-milked shows acidic reaction. This is called first acidity or natural acidity. First acidity is affected by age, species, breed and character of the animal, lactation period diseases and the composition of milk. Milk can't preserve its first acidity. Milk is contaminated by different types of microorganisms due to milking conditions. Milk is a great food source and breeding environment for transmitted bacteria. Especially, milk acid bacteria decompose lactose into energy and lactic acid by secreting enzymes. Lactic acid increases the acidity of milk. Acidity that is formed this way is called developing acidity.

Enzyme activation tests

Catalase test

Catalase enzyme is generally present in every milk and its amount is increased by some physiological and pathological factors. Especially colostrum and milk of animals with mastitis contain larger amount of catalase enzyme. Catalase test is done in order to analyze the state caused by these physiological and pathological factors and especially the properties of milk that is going to be used in cheese production (Kınık et al., 2011).

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Peroxidase test

Peroxidase is one of the enzymes that milk contains naturally. It goes into inactive state in 2,5 minutes at 70°C, in 1,5 seconds at 78°C and in 2,5 seconds at 80°C.

Preservative substances check

Some preservative substances are used to keep milk from spoiling and to increase its resistance. However, usage of these substances are prohibited by law. Most common substances used are some alkaline substances, formaldehyde, hydrogen peroxide, certain antibiotics.

Mastitis check

pH value of milk of animals with mastitis goes over 7 and it can even reach to 9,5 in acute mastitis cases (Ak, 2010). Decrease in susceptibility of milk of animals with mastitis to rennet, meaning clotting of milk taking longer composes a loose clot and makes extraction of whey harder. Excess amount of whey remains in clot and it decreases the amount of dry matter. This negatively effects quality. On the other hand, excess amount of whey proteins that remain in clot are reduced to undesired materials during maturation process and because cheese have a bitter taste.

Analysis data of milk samples collected from certain villages of Biga (Canakkale) region

The milk to be analyzed is taken from milk collection centers. Taking into consideration the size of the container in which the milk was stored, the samples were mixed for five minutes using a stirring bar and samples were taken from tank (Uslu, 2009). The samples are approximately 200 ml, placed in clean and sterile sample containers, sealed well, and labeled. On the label, information such as sample type, place of receipt of the bulb, date of receipt of the sample, temperature grade, what purpose it was received (Demirbaş, 2012) is written. Milk samples were sent to the laboratory between 0°C and 9°C temperatures and protected from sunlight.

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Table 1. Analysis data of Danişment village

Row Number	Fat(%)	Protein (%)	Lactose (%)	YKM (%)	Cryoscopic water amount (%)	FP (°C)	SCC
1	3,95	3,95	4,31	12,01	0	0,546	3466,000
2	3,29	2,87	4,33	10,96	0	0,542	598,000
3	3,45	2,76	4,84	11,54	0	0,554	16,000
4	3,75	2,71	4,61	11,56	0	0,544	125,000
5	3,24	3,31	4,52	11,54	0	0,545	981,000
6	3,82	2,71	4,41	11,36	0	0,552	376,000
7	3,45	2,66	4,61	11,19	0	0,549	536,000
8	3,24	2,82	4,42	10,98	0	0,541	490,000
9	3,29	2,61	4,43	10,81	0	0,551	420,000
10	4,01	2,87	4,84	12,18	0	0,567	280,000
11	3,41	2,86	4,31	11,06	0	0,541	743,000
12	4,19	3,51	4,49	12,66	0	0,552	233,000
13	3,18	2,75	4,72	11,14	0	0,549	207,000
14	4,13	3,05	4,27	11,95	0	0,546	441,000
15	3,88	3,06	4,59	11,98	0	0,553	234,000
16	3,59	3,17	4,59	11,8	0	0,559	1375,000
17	3,57	2,69	4,74	11,47	0	0,549	143,000
18	3,47	2,81	4,53	11,28	0	0,551	717,000
19	4,55	3,11	4,07	12,12	0	0,547	331,000
20	3,23	2,68	4,53	10,92	0	0,546	907,000
21	3,54	3,15	4,54	11,72	0	0,555	884,000
22	3,39	2,54	4,48	10,84	0	0,557	520,000
23	3,82	2,83	4,11	11,21	0	0,539	1375,000
24	3,09	3,31	4,71	11,59	0	0,559	748,000
25	3,08	3,07	4,46	11,14	0	0,544	358,000
26	3,76	2,99	4,86	12,13	0	0,567	478,000
27	3,81	3,16	4,28	11,68	0	0,554	1969,000
28	2,52	3,44	4,37	10,83	0	0,546	418,000
29	3,66	2,96	4,73	11,81	0	0,571	249,000
30	3,94	2,96	4,72	12,11	0	0,555	383,000
31	3,42	2,9	4,44	11,23	0	0,543	976,000
32	3,73	3,34	3,92	11,43	0	0,542	4787,000
33	3,04	2,87	4,42	10,79	0	0,551	986,000
34	3,43	3,12	4,41	11,43	0	0,542	230,000
35	3,78	3,18	4,61	12,03	0	0,555	503,000
36	3,22	2,77	4,69	11,18	0	0,551	1986,000
37	2,89	2,66	4,41	10,45	1,2	0,514	112,000
38 Aggregate	3,49	2,95	4,51	11,42	0	0,551	816,000

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Table 2. Analysis data of Havdan village

Row Number	Fat (%)	YKM (%)	Density (%)	Protein (%)	Lactose (%)	Cryoscopic water amount (%)
1	3,06	8,82	28,49	3,11	3,92	1,9
2	3,11	8,89	28,85	3,08	3,95	1,0
3	3,78	9,08	25,21	2,86	3,52	0
4	3,77	9,47	27,93	3,11	3,87	0
5	3,71	9,49	27,93	3,11	3,87	0
6	3,25	8,87	28,61	3,06	3,92	1,7
7	3,26	9,28	27,29	3,01	3,77	0
8	3,21	9,66	28,82	3,14	3,97	0
9	3,25	9,31	27,38	3,01	3,78	0
10	3,03	9,52	28,33	3,08	3,91	0
11	3,43	9,34	27,51	3,03	3,81	0
12	3,48	9,96	29,92	3,27	4,12	0
13	3,01	9,33	27,59	3,01	3,81	0
14	3,04	8,71	25,21	2,84	3,51	4,0
15	3,52	9,71	28,91	3,17	3,99	0
16	3,75	9,39	27,56	3,08	3,82	0
17	3,44	9,26	27,17	3,01	3,76	0
18	3,68	9,32	27,33	3,03	3,78	0
19	3,26	8,98	26,51	2,92	3,67	1,0
20	3,47	9,65	28,71	3,15	3,96	0
21	3,31	9,62	28,63	3,13	3,95	0
22	3,01	8,82	29,22	3,11	4,01	3,7
23	3,25	8,87	27,15	2,98	3,75	1,5
24	3,29	8,82	28,91	3,16	3,98	1,9
25	3,14	8,76	29,11	3,09	3,98	2,7
26	3,62	9,62	28,57	3,15	3,95	0
27	3,33	9,62	28,65	3,14	3,95	0
28	3,31	9,43	27,91	3,06	3,85	0
29	3,11	8,74	27,09	2,97	3,74	2,3
30	3,08	8,71	26,68	2,88	3,67	2,9
31	3,21	8,73	28,96	3,16	3,99	2,3
32 Aggregate	3,58	9,12	26,31	2,99	3,67	0,8

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Table 3. Analysis data of Güleç village

Row Number	Fat(%)	YKM(%)	Density(%)	Protein(%)	Lactose	Cryoscopic water amount(%)
1	3,65	9,64	28,21	3,23	3,93	0
2	3,55	9,35	27,22	3,09	3,79	0
3	3,54	9,59	27,71	3,26	3,89	0
4	3,11	8,89	26,48	3,01	3,69	2,7
5	3,53	9,07	25,61	3,05	3,57	0
6	3,57	9,26	27,05	3,02	3,75	0
7	3,47	9,41	27,52	3,09	3,82	0
8	3,16	8,87	28,49	3,11	3,92	1,3
9	3,25	8,97	26,19	3,07	3,65	1,0
10	3,74	9,36	27,21	3,08	3,81	0
11	3,79	9,71	28,51	3,24	3,97	0
12	3,62	9,53	28014	3,13	3,91	0
13	3,45	10,12	30,08	3,42	4,18	0
14	3,24	8,82	26,67	3,03	3,72	1,3
15	3,75	9,81	29,21	3,23	4,03	0
16	3,79	9,33	27,31	3,05	3,79	0
17	3,24	8,84	27,42	3,07	3,81	1,5
18	3,62	9,43	27,79	3,07	3,84	0
19	3,06	8,81	26,65	3,08	3,68	1,0
20	3,11	8,78	26,91	3,05	3,73	2,1
21	3,38	9,57	28,43	3,12	3,92	0
22	3,21	8,82	28,31	3,13	3,91	1,0
23	3,07	8,86	28,59	3,07	3,92	1,3
24	3,21	10,21	30,99	3,36	4,25	0
25	3,54	8,97	28,44	3,14	3,93	1,2
26	3,31	9,58	28,49	3,12	3,93	0
27	3,75	9,95	29,73	3,31	4,11	0
28	3,87	9,95	29,81	3,29	4,11	0
29	3,21	9,63	28,71	3,13	3,95	0
30	3,51	8,87	27,32	3,03	3,78	2,3
31	3,49	9,45	27,93	3,08	3,86	0
32	3,41	9,98	30,04	3,28	4,13	0
33	3,51	9,93	29,82	3,26	4,11	0
34	3,45	8,94	25,79	3,07	3,59	2,9
35	3,71	9,46	27,81	3,11	3,86	0
36	3,29	10,16	30,78	3,34	4,23	0
37	3,87	9,54	28,16	3,13	3,91	0
38	3,56	9,53	28,22	3,11	3,91	0
39	3,12	8,87	28,24	3,06	3,88	1,3

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40	3,32	9,93	29,87	3,25	4,11	0	
41	3,25	8,92	26,85	3,02	3,72	1,0	
42	3,27	8,91	27,43	3,12	3,83	1,0	
43	3,85	9,06	26,01	3,05	3,64	0	
44	3,31	8,94	25,91	3,04	3,63	1,2	
45	3,73	9,05	26,13	3,07	3,64	0	
46	3,67	9,35	27,44	3,05	3,81	0	
47	3,11	9,54	28,41	3,09	3,91	0	
48	3,21	9,44	27,98	3,06	3,86	0	
49	3,76	9,31	27,27	3,03	3,78	0	
50	3,67	9,62	28,51	3,15	3,94	0	
51	3,76	9,71	28,83	3,19	3,98	0	
52	3,41	10,13	30,62	3,33	4,21	0	
53	3,78	9,72	28,81	3,19	3,99	0	
54	3,73	9,76	28,79	3,29	4,01	0	
55	3,62	9,83	29,21	3,26	4,04	0	
56	3,79	9,99	29,99	3,31	4,13	0	
57	3,71	9,35	27,32	3,07	3,79	0	
58	3,74	9,19	29,76	3,01	3,72	0	
59	3,76	9,49	27,99	3,11	3,87	0	
60	3,68	9,75	28,73	3,26	3,99	0	
61	3,07	9,68	28,97	3,14	3,98	0	
62	3,28	8,87	27,11	3,08	3,75	1,9	
63	3,09	8,97	29,03	3,16	3,99	1,3	
64	3,47	9,29	26,99	3,06	3,76	0	
65	3,27	8,97	26,81	3,18	3,71	1,3	
66	3,45	8,95	25,55	3,01	3,58	2,5	
67	3,52	8,89	27,34	3,08	3,81	1,0	
68 Aggregate	3,71	9,47	27,79	3,12	3,86	0	SCC 675,000

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Table 4. Analysis data of Gürçeşme village

Row Number	Fat(%)	Protein(%)	Lactose(%)	YKM(%)	Cryoscopic water amount(%)	FP(°C)	SCC
1	4,37	2,47	4,46	11,85	0	0,551	2802,000
2	4,03	2,95	4,43	12,01	0	0,566	2367,000
3	3,59	2,57	4,46	11,16	0	0,547	353,000
4	3,88	2,59	4,84	11,89	0	0,566	1513,000
5	3,75	2,78	4,43	11,51	0	0,549	2456,000
6	4,39	2,64	4,86	12,47	0	0,571	493,000
7	3,66	2,83	4,65	11,61	0	0,556	1595,000
8	2,35	2,46	4,57	10,06	0	0,549	510,000
9	4,11	2,77	4,43	11,82	0	0,551	3381,000
10	3,33	2,97	4,86	11,85	0	0,557	264,000
11	3,66	2,64	4,68	11,51	0	0,555	24,000
12	3,51	2,88	4,56	11,48	0	0,554	956,000
13	3,79	2,96	4,53	11,78	0	0,559	200,000
14	3,23	2,41	5,02	11,25	0	0,578	51,000
15	3,58	2,87	4,73	11,75	0	0,555	2755,000
16 Aggregate	3,71	2,77	4,57	11,62	0	0,561	1575,000

Average values of milk samples gathered from certain villages of Biga (Canakkale) region

In this study, the number of somatic cells and the chemical properties of milk, which is the criterion for whether raw milk produced from different regions is produced in compliance with food safety standards, has been examined.

In this scope, four different regions were selected and raw milk samples were taken from each region. Fat, fat free dry matter, protein, somatic cell count, freezing point, cryoscopic water content, lactose and density values of raw milk samples were examined. The mean and standard errors related to the nutrient content of the milk collected from the four regions studied are given in Table 5.

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Table 5. Average values of milk samples gathered from villages and aggregate

Villages	n	Fat (%)	Protein (%)	Lactose (%)	YKM (%)	Density (%)	FP (°C)	SCC
Danişment	37	3,53	2,96	4,49	11,46	-	0,371	800,000
Güleçköy	67	3,47	3,13	3,87	9,38	28,05	-	-
Havdan	31	3,32	3,06	3,85	9,21	27,94	-	-
Gürçeşme	15	3,68	2,71	4,63	11,6	-	0,157	1314000
Mixed	-	3,62	2,95	4,15	10,40	54.1/2=27,05	1.112/2=0,556	1022000

4. RESULT AND RECOMMENDATIONS

The mean and standard errors for the nutrient content of the milk collected from the four working stocks are given in the Table 6. Accordingly, the village as a whole is a statistically significant source of variation. When the differences between the villagers are examined, milk collected from Havdan village in terms of milk fat ratio shows a statistically lower average value than milk collected from other villages (P <0,05). In terms of milk protein, all the villagers are statistically significantly different from each other (P <0.05). Güleç village has the highest value, while the lowest milk protein average is Gürçesme. In terms of lactose ratio, Güleç and Havdan had a similar average (P> 0.05), whereas Danişment had a significantly higher average lactose ratio (P <0,05) than these villages. The highest mean lactose content was obtained from the milk collected from the Danişment village, which is statistically significantly higher than the average of the other villages (P <0.05).

Table 6. Variance analysis table.

Features	Milk Fat, %		Milk Protein, %		Laktoz, %		YKM %		*P*
Villages	$\bar{X}$	SH	$\bar{X}$	SH	$\bar{X}$	SH	$\bar{X}$	SH	
Danişment	$3,54^{ab}$	0,052	$2,98^{a}$	0,029	$4,50^{a}$	0,028	$11,46^{a}$	0,072	0,0035
Güleç	$3,47^{b}$	0,038	$3,14^{b}$	0,022	$3,88^{b}$	0,022	$9,38^{b}$	0,054	<0,0001
Gürçeşme	$3,68^{a}$	0,082	$2,72^{c}$	0,046	$4,63^{c}$	0,045	$11,60^{a}$	0,114	<0,0001
Havdan	$3,32^{c}$	0,057	$3,06^{d}$	0,032	$3,86^{b}$	0,032	9,22b	0,079	<0,0001

Danişment and Gürçesme, which have values in terms of number of somatic cells, are compared. Accordingly, the difference in SHS between the milk collected from these villages is statistically insignificant (P = 0.4354). The SHS geometric mean of the milk collected from the Danişment village was 496,389 cells / ml whereas the same value was calculated as 660,969 cells / ml in the milk collected from the Gürçesme village.

Average fat rates of milk samples fit the criteria's stated in Raw and Heat-Treated Drinking Milk Communiqué of Turkish Food Codex. Fatless dry matter rates are also fit the criteria's stated in RHTDMC of Turkish Food Codex. Protein contents of milk samples are also fit the criteria with the exception of samples from Gürçeşme village which are lower than expected values. According to RHTDMC of Turkish Food Codex, raw cow milk should

contain 500,000 SCC per ml at maximum. Study shows that SCC values of milk from our villages are above set values.

In order to achieve world standards thresholds of SCC which is taken as important criteria in determination of raw milk quality, the producers of dairy production sector should be made aware of this issue (Kavakoğlu *et al*., 2015).

Analysis data shows that SCC values are higher than they should be. Measures to reduce SCC which has a great importance in determination of raw milk quality and a big role in pricing of milk should be taken. In the research literature shows that every producers aims to produce milk that has low somatic cell count. In order to achieve world standards thresholds, dairy producers should be made aware of SCC, protective veterinary medical science practices and natural organic feed mixtures should be used.

First step of reducing SCC is using protective medical science practices. Mainly, protective medical science is practices that are carried out in order to improve nurture-feeding and housing conditions and without the need to use antibiotics. Paying attention to milking rules, vaccinating, precautions related to cleanliness and hygienic, adding materials that will increase the resilience of breast tissue will reduce SCC. In order to ensure hygiene, a milking machine that works regularly and for each cow a different cloth that is dipped in disinfectant solution and used to wipe the cow's breast before and after the milking should be used. This application is called with the slogan as "a towel for each cow" in order to acquire its prevalence (Ayaşan *et al*.,2011).

Another precaution for reducing SCC is reducing the usage of antibiotics or using natural materials that act as antibiotics. These materials consist of essential oils and other medicinal plants. Mostly essential oils act like antibiotics in the body and halt the growth of microorganisms at certain concentrations. Using medicinal plants such as fenugreek, artichoke leaf and ginseng reduces antibiotic usage to minimum. Adding essential oils such as thyme oil, peppermint oil, rosemary oil, clove oil and cinnamon oil to feeds in certain amounts help reducing antibiotic usage by keeping microorganisms under control. Adding selenium, vitamin E and zinc supplements that will increase body resistance to the feeds is another method of reducing SCC. Another method of reducing antibiotic usage is mannoliglisacharides derived from yeasts. Mannoliglissacharides reduce anitibiotics usage by stimulating the immune system of the body along with preventing attachment of harmful microorganism to the intestines and halting their reproduction.

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IDEAL CONVERGENCE OF DOUBLE INTERVAL VALUED NUMBERS DEFINED BY ORLICZ FUNCTION

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ABSTRACT:

In this paper, we introduce some ideal convergent double interval valued numbers sequence spaces defined by Orlicz function and study different properties of these spaces like completeness, solidity, etc. We establish some inclusion relations among them.

Keywords: *Paranorm; completeness; ideal-convergence; interval numbers; Orlicz function.*

1. INTRODUCTION

The notion of I-convergence was initially introduced by Kostyrko, et. al [10] as a generalization of statistical convergence (see [8],[21]) which is based on the structure of the ideal I of subset of natural numbers $\mathbb{N}$. Kostyrko, et. al [11] gave some of basic properties of I-convergence and dealt with extremal I-limit points. Although an ideal is defined as a hereditary and additive family of subsets of a non-empty arbitrary set X, here in our study it suffices to take I as a family of subsets of $\mathbb{N}$, positive integers, i.e. $I \subset 2^{\mathbb{N}}$, such that $A \cup B \in I$ for each $A, B \in I$, and each subset of an element of I is an element of I.

A non-empty family of sets $F \subset 2^{\mathbb{N}}$ is a filter on $\mathbb{N}$ if and only if $\Phi \notin F$, $A \cap B \in F$ for each $A, B \in F$, and any subset of an element of F is in F. An ideal I is called *non-trivial* if $I \neq \Phi$ and $\mathbb{N} \notin I$. Clearly I is a non-trivial ideal if and only if $F = F(I) = \{\mathbb{N} - A: A \in I\}$ is a filter in $\mathbb{N}$, called the filter associated with the ideal I. A non-trivial ideal I is called *admissible* if and only if $\{\{n\}: n \in \mathbb{N}\} \subset I$. A non-trivial ideal I is maximal if there cannot exist any non-trivial ideal $J \neq I$ containing I as a subset. Further details on ideals can be found in Kostyrko, et.al (see [10]). Recall that a sequence $x = (x_k)$ of points in $\mathbb{R}$ is said to be I-convergent to a real number

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ℓ if $\{k \in \mathbb{N}: |x_k - \ell| \geq \varepsilon\} \in I$ for every $\varepsilon > 0$ ([10]). In this case we write $I - \lim x_k = \ell$. Further details on ideal convergence can be found in [20], [25]. The notion of I-convergence double sequence was initially introduced by Tripathy and Tripathy (see [24]).

Interval arithmetic was first suggested by Dwyer [2] in 1951. Development of interval arithmetic as a formal system and evidence of its value as a computational device was provided by Moore [14] in 1959 and Moore and Yang [15] in 1962. Further works on interval numbers can be found in Dwyer [3], Fischer [9] , Markov [13]. Furthermore, Moore and Yang [16], have developed applications to differential equations.

Chiao in [1] introduced sequence of interval numbers and defined usual convergence of sequences of interval number. Ideal of **N** and the corresponding convergence coincides with the usual convergence. If we take $I = I_\delta = \{A \subseteq \mathbb{N}: \delta(A) = 0\}$ where $\delta(A)$ denote the asymptotic density of the set A. Then I_δ is a non-trivial admissible ideal of $\mathbb{N}$ and the corresponding convergence coincides with the statistical convergence.

Sengönül and Eryilmaz in [22] introduced and studied bounded and convergent sequence spaces of interval numbers and showed that these spaces are complete metric space. Esi in [4], [5] introduced and studied strongly almost λ−convergence and statistically almost λ−convergence of interval numbers and lacunary sequence spaces of interval numbers, respectively. In [7], Esi and Hazarika introduced the difference classes of interval numbers. Recently Esi [6] has studied double sequences of interval numbers.

A set consisting of a closed interval of real numbers x such that $a \leq x \leq b$ is called an interval number. A real interval can also be considered as a set. Thus we can investigate some properties of interval numbers, for instance arithmetic properties or analysis properties.We denote the set of all real valued closed intervals by IR. Any elements of IR is called closed interval and denoted by $\overline{x}$. That is $\overline{x} = \{x \in \mathbb{R}: a \leq x \leq b\}$. An interval number $\overline{x}$ is a closed subset of real numbers [1]. Let x_l and x_r be first and last points of $\overline{x}$ interval number, respectively. For $\overline{x}_1, \overline{x}_2 \in$IR, we have $\overline{x}_1 = \overline{x}_2 \Leftrightarrow x_{1_l}=x_{2_l}, x_{1_r}=x_{2_r}$. $\overline{x}_1 + \overline{x}_2 = \{x \in \mathbb{R}: x_{1_l} + x2l \leq x \leq x1r + x2r$,and if $\alpha \geq 0$, then $\alpha x = x \in \mathbb{R}: \alpha x1l \leq x \leq \alpha x1r$ and if $\alpha < 0$, then $\alpha\overline{x} = \{x \in \mathbb{R}: \alpha x_{1_r} \leq x \leq \alpha x_{1_l}\}$,

$$\overline{x}_1 . \overline{x}_2 = \begin{Bmatrix} x \in \mathbb{R}: \min\{x_{1_l}.x_{2_l}, x_{1_l}.x_{2_r}, x_{1_r}.x_{2_l}, x_{1_r}.x_{2_r}\} \leq x \\ \leq \max\{x_{1_l}.x_{2_l}, x_{1_l}.x_{2_r}, x_{1_r}.x_{2_l}, x_{1_r}.x_{2_r}\} \end{Bmatrix}.$$

In [14], Moore proved that the set of all interval numbers IR is a complete metric space defined by

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$$d(\overline{x}_1, \overline{x}_2) = \max\{|x_{1_l} - x_{2_l}|, |x_{1_r} - x_{2_r}|\}.$$

In the special case $\overline{x}_1 = [a, a]$ and $\overline{x}_2 = [b, b]$, we obtain usual metric of $\mathbb{R}$.

Let us define transformation $f: \mathbb{N} \to \mathbb{R}$ by $k \to f(k) = \overline{x}, \overline{x} = (\overline{x}_k)$. Then $\overline{x} = (\overline{x}_k)$ is called sequence of interval numbers. The $\overline{x}_k$ is called k^{th} term of sequence $\overline{x} = (\overline{x}_k)$. w^i denotes the set of all interval numbers with real terms and the algebraic properties of w^i can be found in [1].

Now we give the definition of convergence of interval numbers:

A sequence $\overline{x} = (\overline{x}_k)$ of interval numbers is said to be convergent to the interval number $\overline{x}_o$ if for each $\varepsilon > 0$ there exists a positive integer k_o such that $d(\overline{x}_k, \overline{x}_o) < \varepsilon$ for all $k \geq k_o$ and we denote it by $\lim_k \overline{x}_k = \overline{x}_o$ [1] .

Thus, $\lim_k \overline{x}_k = \overline{x}_o \Leftrightarrow \lim_k x_{k_l} = x_{o_l}$ and $\lim_k x_{k_r} = x_{o_r}$.

Recall in [17],[12] that an Orlicz function M is continuous, convex, nondecreasing function define for $x > 0$ such that $M(0) = 0$ and $M(x) > 0$. If convexity of Orlicz function is replaced by $M(x + y) \leq M(x) + M(y)$ then this function is called the modulus function and characterized by Ruckle [19]. An Orlicz function M is said to satisfy $\Delta_2 - condition$ for all values u, if there exists $K > 0$ such that $M(2u) \leq KM(u), u \geq 0$. Subsequently, the notion of Orlicz function was used to defined sequence spaces by Tripathy et al [23], Tripathy and Hazarika[26] and many others.

An interval valued double sequence $\overline{x} = (\overline{x}_{k,l})$ is said to be convergent in the Pringsheim's sense or P-convergent to an interval number $\overline{x}_o$, if for every $\varepsilon > 0$, there exists $N \in \mathbb{N}$ such that

$$d(\overline{x}_{k,l}, \overline{x}_o) < \varepsilon \text{for} k, l > N \quad (see\ Pringsheim)$$

and we denote it by $P - \lim \overline{x}_{k,l} = \overline{x}_o$, where $d(\overline{x}_{k,l}, \overline{y}_{k,l})$ is the Hausdorff distance between $\overline{x} = (\overline{x}_{k,l})$ and $\overline{y} = (\overline{y}_{k,l})$. The interval number $\overline{x}_o$ is called the Pringsheim limit of $\overline{x} = (\overline{x}_{k,l})$. More exactly, we say that a double sequence of interval numbers $\overline{x} = (\overline{x}_{k,l})$ converges to a finite interval number $\overline{x}_o$ if $\overline{x}_{k,l}$ tends to $\overline{x}_o$ as both k and l tend to infinity independently of each another. We denote by $\overline{c}^2$ the set of all double convergent interval numbers of double interval numbers.

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The interval number double sequence $\overline{x} = (\overline{x}_{k,l})$ is bounded if and only if there exists a positive number B such that $d(\overline{x}_{k,l}, \overline{0}) < B$ for all k and l. We shall denote all bounded interval number double sequences by $\overline{l}_{\infty}^{2}$. Let $\overline{w}^{2}$ denote the set of all double sequences of interval numbers.

Let $p = (p_{i,j})$ be a double sequence of positive real numbers. If $0 < p_{i,j} \leq \sup_{i,j} p_{i,j} = H < \infty$ and $D = \max(1, 2^{H-1})$, then for $a_{i,j}, b_{i,j} \in \mathbb{R}$ for all $i, j \in \mathbb{N}$, we have $|a_{i,j} + b_{i,j}|^{p_{i,j}} \leq D(|a_{i,j}|^{p_{i,j}} + |b_{i,j}|^{p_{i,j}})$.

2. MAIN RESULTS

In this paper, we define new double sequence spaces for interval sequences as follows.

Let $\mathcal{J}$ be an admissible ideal of $\mathbb{N} \times \mathbb{N}$. Let M be an Orlicz function and $p = (p_{i,j})$ be a double sequence of strictly positive real numbers. We introduce the following sequence spaces:

$$ {}_2\overline{w}^{\mathcal{J}}(M,p) = \left\{ \overline{x} = (\overline{x}_{i,j}) : \left\{ (m,n) \in \mathbb{N} \times \mathbb{N} : \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho} \right) \right]^{p_{i,j}} \geq \varepsilon \right\} \in \mathcal{J}, \right. $$
$$ \left. for\ some \rho > 0, and \overline{x}_o \in \mathbf{IR} \right\}, $$

$$ {}_2\overline{w}_o^{\mathcal{J}}(M,p) = \left\{ \overline{x} = (\overline{x}_{i,j}) : \left\{ (m,n) \in \mathbb{N} \times \mathbb{N} : \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M\left(\frac{d(\overline{x}_{i,j}, \overline{0})}{\rho} \right) \right]^{p_{i,j}} \geq \varepsilon \right\} \in \mathcal{J}, \right. $$
$$ \left. for\ some \rho > 0 \right\} $$

$$ {}_2\overline{w}_{\infty}^{\mathcal{J}}(M,p) = \left\{ \overline{x} = (\overline{x}_{i,j}) : \exists K > 0 s.t. \left\{ (m,n) \in \mathbb{N} \times \mathbb{N} : \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M\left(\frac{d(\overline{x}_{i,j}, \overline{0})}{\rho} \right) \right]^{p_{i,j}} \geq K \right\} \in \mathcal{J}, \right. $$
$$ \left. for\ some \rho > 0 \right\}. $$

and

$$ {}_2\overline{w}_{\infty}(M,p) = \left\{ \overline{x} = (\overline{x}_{i,j}) : \sup_{m,n} \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M\left(\frac{d(\overline{x}_{i,j}, \overline{0})}{\rho} \right) \right]^{p_{i,j}} < \infty, \right. $$
$$ \left. for\ some \rho > 0 \right\}. $$

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Theorem 2.1. *Let* $p=(p_{i,j})$ *be bounded. Then the double sequence spaces* ${}_2\overline{w}^{\mathcal{J}}(M,p)$, ${}_2\overline{w}_o^{\mathcal{J}}(M,p)$ *and* ${}_2\overline{w}_\infty^{\mathcal{J}}(M,p)$ *are linear spaces.*

Proof. It is easy, so omitted it.

Theorem 2.2.*The double sequence spaces* ${}_2\overline{w}^{\mathcal{J}}(M,p)$, ${}_2\overline{w}_o^{\mathcal{J}}(M,p)$ *and* ${}_2\overline{w}_\infty^{\mathcal{J}}(M,p)$ *are paranormed sequence spaces paranormed by*

$$\overline{g}(\overline{x})=\inf\left\{\rho^{\frac{p_{i,j}}{H}}:\sup_{i,j}M\left(\frac{d(\overline{x}_{i,j},\overline{0})}{\rho}\right)\leq 1\right\}$$

where$H=max\left(1,\sup_{i,j}p_{i,j}<\infty\right)$.

Proof. Clearly $\overline{g}(\overline{0})=0,\overline{g}(\overline{x})=\overline{g}(-\overline{x})$. Let $\overline{x}=(\overline{x}_{i,j}),\overline{y}=\left(\overline{y}_{i,j}\right)\in$ ${}_2\overline{w}^{\mathcal{J}}(M,p)$. Then there exist some $\rho_1>0$ and $\rho_2>0$ such that

$$\sup_{i,j}M\left(\frac{d(\overline{x}_{i,j},\overline{0})}{\rho_1}\right)\leq 1\text{and}\sup_{i,j}M\left(\frac{d\left(\overline{y}_{i,j},\overline{0}\right)}{\rho_2}\right)\leq 1.$$

Let $\rho=\rho_1+\rho_2$, then we have

$$\sup_{i,j}M\left(\frac{d\left(\overline{x}_{i,j}+\overline{y}_{i,j},\overline{0}\right)}{\rho}\right)$$

$$\leq\frac{\rho_1}{\rho_1+\rho_2}\sup_{i,j}M\left(\frac{d(\overline{x}_{i,j},\overline{0})}{\rho_1}\right)+\frac{\rho_2}{\rho_1+\rho_2}\sup_{i,j}M\left(\frac{d\left(\overline{y}_{i,j},\overline{0}\right)}{\rho_2}\right)$$

$$\leq 1.$$

Now

$$\overline{g}(\overline{x}+\overline{y})=\inf\left\{(\rho_1+\rho_2)^{\frac{p_{i,j}}{H}}:\sup_{i,j}M\left(\frac{d\left(\overline{x}_{i,j}+\overline{y}_{i,j},\overline{0}\right)}{\rho}\right)\leq 1\right\}$$

$$\leq\inf\left\{\rho_1^{\frac{p_{i,j}}{H}}:\sup_{i,j}M\left(\frac{d(\overline{x}_{i,j},\overline{0})}{\rho_1}\right)\leq 1\right\}$$

$$+\inf\left\{\rho_2^{\frac{p_{i,j}}{H}}:\sup_{i,j}M\left(\frac{d\left(\overline{y}_{i,j},\overline{0}\right)}{\rho_2}\right)\leq 1\right\}$$

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$$= \overline{g}(\overline{x}) + \overline{g}(\overline{y}).$$

Let $\beta \in \mathbb{R}$, then the continuity of the product follows from the following inequality:

$$\overline{g}(\beta\overline{x}) = \inf\left\{\rho^{\frac{p_{i,j}}{H}} : \sup_{i,j} M\left(\frac{d(\beta\overline{x}_{i,j},\overline{0})}{\rho}\right) \leq 1\right\}$$

$$= \inf\left\{(|\beta|r)^{\frac{p_{i,j}}{H}} : \sup_{i,j} M\left(\frac{d(\overline{x}_{i,j},\overline{0})}{r}\right) \leq 1\right\}$$

where $\frac{1}{r} = \frac{|\beta|}{\rho}$. This completes the proof.

Theorem 2.3. *The double sequence spaces* ${}_2\overline{w}^{\mathcal{J}}(M,p)$, ${}_2\overline{w}_o^{\mathcal{J}}(M,p)$, ${}_2\overline{w}_\infty^{\mathcal{J}}(M,p)$ *and* ${}_2\overline{w}_\infty(M,p)$ *are complete paranormed spaces, paranormed by* $\overline{g}$ *defined by Theorem 2.2.*

Proof. Let $\left(\overline{x}_{i,j}^s\right)$ be a Cauchy sequence in ${}_2\overline{w}_\infty(M,p)$. Then $\overline{g}\left(\left(\overline{x}_{i,j}^s\right) - \left(\overline{x}_{i,j}^t\right)\right) \to 0$ as $s,t \to \infty$. For given $\varepsilon > 0$, choose $r > 0$ and $x_o > 0$ be such that $\frac{\varepsilon}{rx_o} > 0$ and $M\left(\frac{rx_o}{2}\right) \geq 1$. Now $\overline{g}\left(\left(\overline{x}_{i,j}^s\right) - \left(\overline{x}_{i,j}^t\right)\right) \to 0$ as $s,t \to \infty$ implies that there exists $n_o \in \mathbb{N}$ such that

$$\overline{g}\left(\left(\overline{x}_{i,j}^s\right) - \left(\overline{x}_{i,j}^t\right)\right) < \frac{\varepsilon}{rx_o} \text{ for all } s,t \geq n_o.$$

Then

$$\inf\left\{\rho^{\frac{p_{i,j}}{H}} : \sup_{i,j} M\left(\frac{d\left(\overline{x}_{i,j}^s - \overline{x}_{i,j}^t, \overline{0}\right)}{\rho}\right) \leq 1\right\} < \frac{\varepsilon}{rx_o}. \tag{2.1}$$

Now from (2.1), we have

$$M\left(\frac{d\left(\overline{x}_{i,j}^s - \overline{x}_{i,j}^t, \overline{0}\right)}{\rho}\right) \leq 1 \leq M\left(\frac{rx_o}{2}\right)$$

$$\Rightarrow \frac{d\left(\overline{x}_{i,j}^s - \overline{x}_{i,j}^t, \overline{0}\right)}{\overline{g}\left(\left(\overline{x}_{i,j}^s\right) - \left(\overline{x}_{i,j}^t\right)\right)} < \frac{rx_o}{2} \cdot \frac{\varepsilon}{rx_o} = \frac{\varepsilon}{2}.$$

This implies that $\left(\overline{x}_{i,j}^s\right)$ is a Cauchy sequence of real numbers. Let $\lim_{s\to\infty} \overline{x}_{i,j}^s = \overline{x}_{i,j}$. Using continuity of M, we have

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$$\limsup_{t\to\infty}{}_{i,j} M\left(\frac{d\left(\overline{x}_{i,j}^{s}-\overline{x}_{i,j}^{t},\overline{0}\right)}{\rho}\right)\leq 1$$

$$\Rightarrow \sup_{i,j} M\left(\frac{d(\overline{x}_{i,j}^{s}-\overline{x}_{i,j},\overline{0})}{\rho}\right)\leq 1.$$

Let $s\geq n_o$, then taking infimum of such $\rho's$, we have $\overline{g}\left(\left(\overline{x}_{i,j}^{s}\right)-\left(\overline{x}_{i,j}\right)\right)<\varepsilon$. Thus $\left(\overline{x}_{i,j}^{s}\right)-\left(\overline{x}_{i,j}\right)\in_2 \overline{w}_{\infty}(M,p)$. By linearity of the double space ${}_2\overline{w}_{\infty}(M,p)$, we have $\left(\overline{x}_{i,j}\right)\in_2 \overline{w}_{\infty}(M,p)$. Hence ${}_2\overline{w}_{\infty}(M,p)$ is complete. This completes the proof.

Theorem 2.4. (a) ${}_2\overline{w}^{\mathcal{J}}(M,p)\subset {}_2\overline{w}_{\infty}(M,p)$,

(b) ${}_2\overline{w}_o^{\mathcal{J}}(M,p)\subset {}_2\overline{w}_{\infty}(M,p)$.

Proof. It is easy, so omitted.

Theorem 2.5. *The double sequence spaces* ${}_2\overline{w}^{\mathcal{J}}(M,p)$ *and* ${}_2\overline{w}_o^{\mathcal{J}}(M,p)$ *are nowhere dense subsets of* ${}_2\overline{w}_{\infty}(M,p)$.

Proof. The proof is obvious in view of Theorem 2.3 and Theorem 2.4.

Theorem 2.6. (a) *If* $0<inf_{i,j}p_{i,j}\leq p_{i,j}<1$, *then* ${}_2\overline{w}^{\mathcal{J}}(M,p)\subset {}_2\overline{w}^{\mathcal{J}}(M)$,

(b) *If* $1<p_{i,j}<sup_{i,j}p_{i,j}<\infty$, *then* ${}_2\overline{w}^{\mathcal{J}}(M)\subset {}_2\overline{w}^{\mathcal{J}}(M,p)$,

(c) *If* $0<p_{i,j}\leq q_{i,j}<\infty$ *and* $\left(\frac{q_{i,j}}{p_{i,j}}\right)$ *is bounded, then* ${}_2\overline{w}^{\mathcal{J}}(M,p)\subset {}_2\overline{w}^{\mathcal{J}}(M,q)$.

Proof. The first part of the result follows from the relation

$$\left\{(m,n)\in\mathbb{N}\times\mathbb{N}:\frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n} M\left(\frac{d(\overline{x}_{i,j},\overline{x}_o)}{\rho}\right)\geq\varepsilon\right\}$$

$$\subseteq\left\{(m,n)\in\mathbb{N}\times\mathbb{N}:\frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M\left(\frac{d(\overline{x}_{i,j},\overline{x}_o)}{\rho}\right)\right]^{p_{i,j}}\geq\varepsilon\right\}$$

and the second part of the result follows from the relation

$$\left\{(m,n)\in\mathbb{N}\times\mathbb{N}:\frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M\left(\frac{d(\overline{x}_{i,j},\overline{x}_o)}{\rho}\right)\right]^{p_{i,j}}\geq\varepsilon\right\}$$

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$$\subseteq \left\{(m,n) \in \mathbb{N} \times \mathbb{N} \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} M\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right) \geq \varepsilon\right\}.$$

This completes the proof.

The proof of the part three is easy, so omitted.

Theorem 2.7.(a) *If* $0 < inf_{i,j} p_{i,j} \leq p_{i,j} < 1$, *then* ${}_2\overline{w}_0^{\mathcal{I}}(M,p) \subset {}_2\overline{w}_0^{\mathcal{I}}(M)$,

(b) *If* $1 < p_{i,j} < sup_{i,j} p_{i,j} < \infty$, *then* ${}_2\overline{w}_0^{\mathcal{I}}(M) \subset {}_2\overline{w}_0^{\mathcal{I}}(M,p)$,

(c) *If* $0 < p_{i,j} \leq q_{i,j} < \infty$ *and* $\left(\frac{q_{i,j}}{p_{i,j}}\right)$ *is bounded, then* ${}_2\overline{w}_0^{\mathcal{I}}(M,p) \subset {}_2\overline{w}_0^{\mathcal{I}}(M,q)$.

Proof of the result follows from the Theorem 2.6.

Theorem 2.8. *Let* M_1 *and* M_2 *be two Orlicz functions. Then*

$${}_2\overline{w}^{\mathcal{I}}(M_1,p) \cap {}_2\overline{w}^{\mathcal{I}}(M_2,p) \subset {}_2\overline{w}^{\mathcal{I}}(M_1 + M_2, p).$$

Proof. Let $(\overline{x}_{i,j}) \in {}_2\overline{w}^{\mathcal{I}}(M_1,p) \cap {}_2\overline{w}^{\mathcal{I}}(M_2,p)$. Then for every $\varepsilon > 0$ we have

$$\left\{(m,n) \in \mathbb{N} \times \mathbb{N}: \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho_1}\right)\right]^{p_{i,j}} \geq \varepsilon\right\} \in \mathcal{I}, \ \ for some \rho_1 > 0$$

and

$$\left\{(m,n) \in \mathbb{N} \times \mathbb{N}: \frac{1}{mn} \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M_2\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho_2}\right)\right]^{p_{i,j}} \geq \varepsilon\right\} \in \mathcal{I}, \ \ for some \rho_2 > 0.$$

Let $\rho = \max\{\rho_1, \rho_2\}$. The result follows from the following inequality

$$\sum_{i=1}^{m} \sum_{j=1}^{n} \left[(M_1 + M_2)\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right]^{p_{i,j}}$$

$$\leq D\left(\sum_{i=1}^{m} \sum_{j=1}^{n} \left[M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho_1}\right)\right]^{p_{i,j}} + \sum_{i=1}^{m} \sum_{j=1}^{n} \left[M_2\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho_2}\right)\right]^{p_{i,j}}\right).$$

This completes the proof.

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Theorem 2.9. *Let M_1 and M_2 be two Orlicz functions. Then*

$$ {}_2\overline{w}^{\mathcal{I}}(M_1, p) \subset_2 \overline{w}^{\mathcal{I}}(M_2 \circ M_1, p). $$

Proof. Let $\inf p_{i,j} = H_0$. For given $\varepsilon > 0$, we first choose $\varepsilon_0 > 0$ such that $\max\{\varepsilon_0^H, \varepsilon_0^{H_0}\} < \varepsilon$. Now using the continuity of M_2 choose $0 < \delta < 1$ such that $0 < t < \delta$ implies $M_2(t) < \varepsilon_0$. Let $(\overline{x}_{i,j}) \in_2 \overline{w}^{\mathcal{I}}(M_1, p)$. Now from the definition of ${}_2\overline{w}^{\mathcal{I}}(M_1, p)$, for some $\rho > 0$

$$ \overline{A}(\delta) = \left\{(m,n) \in \mathbb{N} \times \mathbb{N}: \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right]^{p_{i,j}} \geq \delta^H\right\} \in \mathcal{I}. $$

Thus if $(m,n) \notin \overline{A}(\delta)$ then we have

$$ \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right]^{p_{i,j}} < \delta^H $$

$$ \Rightarrow \sum_{i=1}^{m}\sum_{j=1}^{n}\left[M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right]^{p_{i,j}} < mn\delta^H $$

$$ \Rightarrow \left[M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right]^{p_{i,j}} < \delta^H, for all i,j = 1,2,3 \ldots $$

$$ \Rightarrow M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right) < \delta, for all i,j = 1,2,3 \ldots. $$

Hence from above inequality and using continuity of M_2, we must have

$$ M_2\left(M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right) < \varepsilon_0, for all i,j = 1,2,3 \ldots. $$

which consequently implies that

$$ \sum_{i=1}^{m}\sum_{j=1}^{n}\left[M_2\left(M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right)\right]^{p_{i,j}} < mn\max\{\varepsilon_0^H, \varepsilon_0^{H_0}\} < mn\varepsilon $$

$$ \Rightarrow \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M_2\left(M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right)\right]^{p_{i,j}} < \varepsilon. $$

This shows that

$$ \left\{(m,n) \in \mathbb{N} \times \mathbb{N}: \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M_2\left(M_1\left(\frac{d(\overline{x}_{i,j}, \overline{x}_o)}{\rho}\right)\right)\right]^{p_{i,j}} \geq \varepsilon\right\} \subset \overline{A}(\delta) $$

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and so belongs to $\mathcal{J}$. This completes the proof.

Theorem 2.10. *Let M_1 and M_2 be two Orlicz functions. Then*

(a) ${}_2\overline{w}_0^{\mathcal{J}}(M_1,p) \cap {}_2\,\overline{w}_0^{\mathcal{J}}(M_2,p) \subset {}_2\,\overline{w}_0^{\mathcal{J}}(M_1+M_2,p)$;

(b) ${}_2\overline{w}_0^{\mathcal{J}}(M_1,p) \subset {}_2\,\overline{w}_0^{\mathcal{J}}(M_2\circ M_1,p)$.

The proof of the theorem follows from the Theorems 2.8 and 2.9.

Theorem 2.11. *Let M_1 and M_2 be two Orlicz functions satisfying Δ_2-condition. If $\beta = lim_{t\to\infty}\frac{M_2(t)}{t} \geq 1$, then*

(a) ${}_2\overline{w}_0^{\mathcal{J}}(M_1,p) =_2 \overline{w}_0^{\mathcal{J}}(M_2\circ M_1,p)$,

(b) ${}_2\overline{w}^{\mathcal{J}}(M_1,p) =_2 \overline{w}^{\mathcal{J}}(M_2\circ M_1,p)$.

Proof. It is easy, so omitted.

Theorem 2.12. *The double sequence space ${}_2\overline{w}^{\mathcal{J}}(M,p)$, ${}_2\overline{w}_o^{\mathcal{J}}(M,p)$, ${}_2\overline{w}_\infty^{\mathcal{J}}(M,p)$ and ${}_2\overline{w}_\infty(M,p)$ are solid as well as monotone.*

Proof. We give the proof for only ${}_2\overline{w}_o^{\mathcal{J}}(M,p)$. The others can be proved similarly. Let $\overline{x} = \left(\overline{x}_{i,j}\right) \in {}_2\,\overline{w}_o^{\mathcal{J}}(M,p)$ and $\left(\alpha_{i,j}\right)$ be a scalar sequence such that $\left|\alpha_{i,j}\right| \leq 1$ for all $i,j \in \mathbb{N}$. Then for every $\varepsilon > 0$ we have

$$\left\{(m,n) \in \mathbb{N}\times\mathbb{N}: \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M\left(\frac{d(\alpha_{i,j}\overline{x}_{i,j},\overline{0})}{\rho}\right)\right]^{p_{i,j}} \geq \varepsilon\right\}$$

$$\subseteq \left\{(m,n) \in \mathbb{N}\times\mathbb{N}: \frac{E}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\left[M\left(\frac{d(\overline{x}_{i,j},\overline{0})}{\rho}\right)\right]^{p_{i,j}} \geq \varepsilon\right\} \in \mathcal{J},$$

where $E = \max\{1, |\alpha_{k,l}|^H\}$. Hence $(\alpha\overline{x}) \in {}_2\,\overline{w}_o^{\mathcal{J}}(M,p)$. This completes the proof.

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