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Research Article

Evaluation of Rabbit Skin Gelatin and Preparation of Yogurt using Rabbit Skin Gelatin

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Abstract: TThe purpose of this research is to know the quality of rabbit skin gelatin soaked in the concentration of different acetic acid as well as its application in yogurt making. Compared to the use of the Arab Gum and CMC (Carboxymethyl Cellulose) The material used in this study was the rabbit skin type Rex male. The treatment of this research is the immersion of acetic acid with 2 %; 3 %; 4 %; 5 % concentration. The results showed that concentrations of acetic acid solution in the process of soaking of acid extract gave a very significant effect ($p < 0.01$) toward yield, pH, protein, fat, viscosity, gel strength, the melting point of rabbit skin gelatin. Application of rabbit skin gelatin on yogurt making gives better yogurt quality compared with the addition of Arabic Gum or CMC. The results showed that the various stabilizers in the yogurt-making process give significant effect ($p < 0.01$) to the resulting pH, The power of the water belt, Synergized, Viscosities of yogurt. However, the various stabilizers in the yogurt-making process give a non-significant effect ($p > 0.05$) to the resulting acidity of yogurt. The conclusion to immersion acetic acid with a concentration of up to 5% will improve the quality of the rabbit skin gelatin and the use of rabbit skin gelatin by 0.8 % as a stabilizer in the manufacture of yogurt produces a better-quality yogurt compared to the Arabic Gum and CMC (Carboxymethyl Cellulose).

Keywords: Quality, Gelatin, Rabbit skin, Yogurt, Acetic acid

1. INTRODUCTION

Gelatin is a polypeptide extracted from the animal tissue of collagen found in bones, skin, and connective tissues [1]. Gelatin is obtained through partial hydrolysis of collagen. When collagen is treated with acids or bases and is followed by heat, the fibrous structure of collagen is broken down irreversibly and produces gelatin [2].

In the food industry, gelatin serves as a stabilizer, thickener, emulsifier, jelly-forming, water-binding, control, and food wrapping (edible coating). Gelatin is also used as a stabilizer, particularly in dairy products [3], and as a fat substitute that can be used to reduce the energy content of food without negative taste [4].

The quality of gelatin is influenced by the gelatin manufacturing process stages such as swelling, extraction, precipitation (extraction result filtering), and drying. The swelling usually uses a solution of acids, bases, or acids and bases. The type and concentration of the acidic solution affect the resulting gelatin properties. This is because immersion is carried out in a relatively shorter acid process of 3 – 4 weeks compared to the alkaline process of about 3 months [5]. The yield and quality of gelatin produced depend on the extraction method carried out on collagen [6].

Gelatin can be made from various types of skin, including fish skin [7], gelatin from chicken leg skin [8], gelatin from goat skin [9], and gelatin from leather shaving waste in the industry [10]. One of the processes that determine the quality of gelatin is the hydrolysis of the skin. Dry cowhide which was hydrolyzed using 1% HCl solvent could produce 65.75% gelatin yield [11]. Meanwhile, hydrolyzed pork skin aged 7 months with 2% acetic acid to produce optimum gelatin production [12]. Optimal goat skin gelatin production was produced

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by soaking for 4 days and using a weak base $Ca(OH)_2$ 9 % (v / v) [9].

In addition, the advantages of the acid process include the preparation of raw materials only require a relatively short time and cheaper cost. The acetic acid solution can turn a triple helix Collagen fiber into a single chain [13]. The concentration of the acetic acid solution also affects the amount of collagen dissolved during the extraction process [14].

Based on this, this study specifically examines the quality of gelatin derived from rabbit skin soaked with different acetic acid concentrations and the application of using rabbit skin gelatin in making yogurt.

2. MATERIALS AND METHODS

The main ingredient of the research uses 16 local male rabbit skins. Rabbit skin material is taken from the rabbit farm in Batu Malang.

2.1 Gelatin extract

The manufacturing of skin rabbit gelatin is carried out by extraction through the acidic process which according to the method of Said *et al.* (2011), the process is as follows: the skin of rabbits that have been clean from the remnants of fat and meat stuck, cut small-sized approximately 1 cm x 1 cm. The skin that has been cut into small pieces is weighed, then to be continued with the immersion process, which is by dissolving CH_3COOH 2 % (v /v); 3 % (v/v); 4 % (v/v); 5 % (v/v) each. Rabbit skin is soaked in the CH_3COOH solution, during the immersion process, stirring is carried out. After the soaking process is finished the skin is washed back with flowing water several times until the washing water has a pH of around 6, after that the extraction was carried out at 65 °C for 3 h. The extraction product is filtered using a gelatin filter cloth, which is obtained and then dried. Before drying is performed at the temperature of 60 °C for 5 h, cooled in a refrigerator 5-10 ºC for 30 min, then dried using an oven 60 °C until dry. The formed gelatin product is then ground by using a blender, packaged in a vacuum plastic container, and stored in a desiccator for the testing process of gelatin quality.

2.2 Yogurt Making stage

The process of making yogurt began by dissolving the skim milk 14 % (14 %, b/v), then adding stabilizer (rabbit skin Gelatin; The Arabic Gum and CMC), then pasteurized at 72 °C for 15 min and are lowered in temperatures up to 43 ºC. Then the milk is inoculated with the pre-prepared starter as much as 3 % (v/v) of starter mixture of L. bulgaricus, S. thermophillus, and L. acidophilus. After that, it was incubated at 41 °C for 4 h. Later, the resulting yogurt is tested for quality. The Arabic Gum is produced from the Acacia sp. tree sap.

The Arabic Gum has Arabinogalactan protein (AGP) and glycoprotein (Gp) cluster which acts as an emulsifier and a coagulate. CMC (Carboxymethyl cellulose) is a derivative anionic polyelectrolyte of cellulose that acts as a water binder, an emulsion stabilizer thickener.

2.3 Physical and Chemical analysis

The quality measurement of rabbit skin gelatin consists of the viscosity of rabbit skin gelatin which is measured by [15]. Degrees of acidity (pH) are measured by [29]. Proximate analysis, namely moisture content, protein levels, and fat levels [16]. The yield was measured by [1]. Moderate quality measurement of yogurt is given consisting of the acidity using titration methods [16], The Power of water [17], and Sineresis [23].

2.4 Statistical Analysis

The collected data were analyzed by analysis of variance (ANOVA) and continued with the Duncan Multiple Range Test (DMRT) [33].

3. RESULTS AND DISCUSSION

3.1 Quality of Rabbit Skin Gelatin

The results of the data analysis of the physicochemical quality of the rabbit skin can be seen in Table 1.

From table 1, it also shows that the higher the concentration of acetic acid, will cause decreased water content of gelatin, this is due to acid ingredients breaking down the structure of amino acids that

compose the skin proteins so that the structure of protein becomes very weak which resulting in a denaturation process. The denaturation process caused the alteration of molecules and the amount of water bound to become weaker and decreased [18]. This resulted in water molecules being easily loose so that when the drying process of the water content value of gelatin decreased.

The physical quality results in the form of rabbit skin gelatin yield are shown that various concentrations of acetic acid solution in the process of soaking of acid extract for 48 h gave a very significant effect ($p < 0.01$) toward yielded rabbit skin gelatin. The yield produced depends on the extraction process used during the manufacture of gelatin [19]. An increasingly large yield indicates that the production process becomes increasingly efficient. The research conducted resulted in a very high range of yield (11.15 – 13.07 %) and higher than the lower range of research results [6], at 5.17 - 6.42 %. The occurrence of increased yield relates to the sheer amount of collagen converted and the transformation into gelatin. In the use of the 2-5 % acetic acid extraction material used in this study showed the onset of improvement effect in loosening the bonding bonds of collagen constituent molecules [19].

The physical quality of the pH of rabbit skin gelatin indicates that the various concentrations of acetic acid solutions in the extraction process for 48 h give a very significant effect ($p < 0.01$) toward the pH of skin gelatin of the resulting rabbit skin. The pH value is also affected by the gelatin manufacturing process due to the interaction process during the extraction process. This affects the components of the skin's collagen fibers. The moment collagen fibers will undergo a process of swelling so that there is a decline in the internal cohesion properties of the skin fibers [20]. The structure of the amino acid bond in the collagen molecule undergoes and the curing material is trapped in the bond and does not dissolve during the neutralization process that will directly affect the final PH value of the resulting gelatin. It is suspected that the neutrality process (washing) of raw materials before heading to the extraction stage runs less than perfect, so the factors that cause the deposition of acid material into the structure of less collagen can be minimized. The neutralization process is a very important process to provide more stable conditions in the intermolecular and intramolecular binding of the skin's constituent proteins [20].

The protein levels of rabbit skin gelatin showed that various concentrations of acetic acid solutions in the extract process for 48 h gave a very significant effect ($p < 0.01$) on the protein levels of the resulting rabbit skin gelatin. High protein levels, associated with the physical properties of other gelatin, the strength of the gel and viscosity. The length of the extraction time and its concentration caused the collagen fibers to shrink, causing the collagen structure to break down into irregular structures and would eventually undergo a dissolving process [13]. The increasing levels of protein are also associated with changes in the number of structures of the amino acids that compose collagen proteins. The increased concentration of extraction materials leads to more and more binding amino acids which

Table 1. The Quality of Rabbit Skin Gelatin with Different Acetic Acid Concentration

Treatment	Water content (%)	Yield (%)	pH	Protein levels (%)	Fat content (%)	Viscosities (CP)	Gel strength (g/Bloom)	Melting Point (°C)
2%	10.59^{a}	11.15^{a}	2.97^{a}	84.21^{a}	2.65^{a}	5.14^{a}	194.01^{a}	20.60^{a}
3%	9.51^{b}	12.11^{b}	4.24^{b}	85.34^{b}	1.49^{b}	5.35^{ab}	205.03^{a}	21.50^{ab}
4%	9.80^{c}	11.24^{a}	4.73^{b}	85.41^{b}	1.65^{b}	5.28^{ab}	205.44^{a}	22.60^{b}
5%	8.23^{d}	13.07^{c}	4.94^{C}	87.55^{c}	0.77^{c}	5.55^{b}	245.52^{b}	22.60^{b}

Description: Different superscripts indicate the presence of noticeable differences ($P < 0.01$).

are more and more proteins that dissolve during the extraction process [21]. Heating during the extraction process will make it easier for collagen to undergo a dissolving or solubilization process.

The fat content of the rabbit skin gelatin showed that the various concentrations of acetic acid solution in the extract process for 48 h gave a very significant effect ($p < 0.01$) toward the resulting fat content of the rabbit skin gelatin. High-quality Gelatin is characterized by low-fat content. The quality requirement of gelatin is to have 5 % fat content [23]. The use of list acid as a soaking ingredient in this study is closely related to the amount of gelatin fat levels produced. The skin as a raw material of gelatin has a fat bound with protein (lipoprotein) [24], added that while soaking using acids, protein structure breaks down in its crosslinking [25]. Partial protein breakdown occurs during the immersion process, which is the process of dissolving the protein molecule so that the possibility of fat molecules bound by the protein molecules also undergo a process of dissolving and being wasted when washing post-extraction. It will affect the resulting fat level of gelatin [21].

The physical quality of the viscosity of rabbit skin gelatin suggests that the various concentrations of acetic acid solutions in the immersion process for 48 h gave a very significant effect ($p < 0.01$) to the viscosity of the resulting rabbit skin gelatin. The increased concentration of acetic acid in immersion in the production process of gelatin can increase the viscosity value. This is because acetic acid has been breaking down the amino acid peptide bond into a very short molecular chain so that the viscosity increases. Soaking with acetic acid can also increase the viscosity value when able to break down the peptide bonds in precise bonding with longer molecules.

The physical qualities of the body of the gel strength of a rabbit skin gelatin showed that various concentrations of acetic acid solutions in the immersion process for 48 h gave a very significant effect ($p < 0.01$) toward the strength of the rabbit skin Gelatin gel Generated. The strength of the gel is one of the important physical properties of the gelatin because the gel strength indicates the ability of gelatin in the formation of gels [1]. Gel formation occurs due to the development of gelatin molecules at the time of heating. The heat will open the bonds to the gelatin molecule and the original free fluid flows into a viscous solution. The solution will form the gel perfectly if it is stored at a cold temperature (10 ºC) for 17 ± 2 h [22]. The results of the gel strength measurement seen in Table 1, can be noted that the highest rabbit skin gelatin gel strength is 245.5 bloom, the value is lower than the commercial gelatin is 328.57 bloom [5]. Laboratory standard gelatin does not form a gel, this is because the function of the gelatin is not as a material forming gel (gelling agent) but only as a blocking agent so that the strength of the gel is not so important for the product [26]. Mammal gelatin has a higher gel strength than the strength of the fish gelatin gel [27]. The strength of the gel is influenced by acids, alkalis, and heat that will damage the gelatin structure so that the gel is not formed [1]. The strength of the gelatin gel is strongly influenced by the concentration of gelatin, pH, temperature, and incubation time [29].

The physical quality of the melting point of the skin gelatin suggests that the various concentrations of acetic acid solutions in the immersion process for 48 h gave a very significant effect ($p < 0.01$) toward the melting point of the resulting rabbit skin gelatin. The low melting point of a rabbit skin gelatin produced is caused because the melting point is strongly influenced by the concentration of soaking materials in the solution, pH, and the magnitude of the molecular gelatin [29]. A low gelatin melting point is caused by low content of amino acids glycine and hydroxypropyl in gelatin, resulting in the loss of hydrogen from gelatin to water in solution. The melting point of rabbit skin gelatin is the highest of 22.6º C, still included in the standard range of melting point temperature of gelatin in general [30]. The gelatin product is a product that is at a temperature < 35 ºC has undergone melting and can melt in the mouth [32].

3.2 Application of Rabbit Skin Gelatin on Yogurt

The result of data analysis of the physicochemical quality of yogurt-given rabbit skin can be seen in table 2.

The quality of the acidity of yogurt as seen in table 2, shows that the various stabilizers in the yogurt making process give non significant effect

Table 2. Yogurt Quality with the addition of various stabilizing materials (Gelatin Rabbit skin, Gum Arabic and CMC)

Treatment	Acidity (%)	pH	Water connective Power (%)	Synergized (%)	Viscosities (CP)
Gelatin Skin Rabbit	0.87[a]	4.37[a]	85.34[a]	0.0085[a]	5.35[a]
Arabian Gum	0.87[a]	4.04[b]	85.21[a]	0.0136[b]	5.28[a]
CMC	0.86[a]	4.03[b]	83.41[b]	0.0265[b]	5.10[b]

Description: Different superscripts indicate the presence of noticeable differences ($p < 0.01$)

($p > 0.05$) to the resulting acidity of yogurt, it is because of the acidity of the Yogurt is due to the breakdown of lactose by lactic acid bacteria, not because of the use of stabilizing material. Gelatin is also a nonpolar neutral stabilizer that cannot release or accept protons so that their existence does not affect nonpolar which has a closed chemical structure, i.e. it does not have a base functional group in its side chain so it cannot accept or release protons and does not affect the acidity of yogurt.

The yogurt pH shows that various stabilizing materials in the yogurt making process give a very noticeable effect ($p < 0.01$) to the resulting pH of yogurt. The giving of rabbit skin gelatin produces a higher yogurt pH than the addition of the Arab Gum and CMC, this is because the value of yogurt pH is caused by a decrease in the amount of H+ ions triggered by a decrease in the total number of acids. The concentration of acid contained in the fermentation product affects the pH value of the concentration of lactic acid hydrogen ions will be followed by increasing the concentration of hydrogen ions so that the pH value decreases, or vice versa [31].

In general, yogurt has a PH value in the range of 4.0 to 4.6 obtained from the fermentation process by using the cultures of Lactobacillus bulgaricus and Streptococcus thermophilus with incubation at a temperature of 43 °C (3 h) [2].

The power of the water belt indicates that the addition of a variety of stabilizer materials gives a very noticeable effect ($p < 0.01$) toward the supply of yogurt water. Power bunch of yogurt water given gelatin rabbit skin is higher than the one added with the Arabic gum or CMC, this is due to rabbit skin gelatin can increase the connective power of yogurt water by preventing the occurrence of hydrogen bonds between the casein molecule and lactic acid and enhance the hydrophilic properties of proteins. Gelatin as a stabilizer can also increase the connective water of yogurt by preventing the occurrence of hydrogen bonds between the casein molecules between the casein and lactic acid molecules, as well as triggering the occurrence of the casein ion payload and maintaining the bond between the protein molecules and the negative casein molecular tendencies to bind with positively charged lactic acid Through hydrogen bonds can be prevented [26].

Synergized that the various additions to the stabilizer have a very noticeable effect ($p < 0.01$) on the synergy of yogurt, this is because the addition of rabbit skin gelatin is a stabilizing material to reduce the synergy as well as a water binder by enhancing the hydrophilic properties of proteins.

From the viscosity, it shows that various additives have a very significant effect ($p < 0.01$) on the viscosity of yogurt, this is because rabbit skin gelatin is able to bind water so that the water molecules are trapped in the gel structure that has been formed. Water that was previously outside the granules and free to move, with the emergence of rabbit skin gelatin becomes unable to move anymore because it is absorbed and bound to the gelatin beads so that the state of the solution

becomes thick, resulting in an increase in viscosity.

4. CONCLUSION

The soaking of acetic acid with a concentration of up to 5 % will improve the quality of rabbit skin gelatin. The results of the application of rabbit skin gelatin as a stabilizer in the manufacture of yogurt showed that the use of rabbit skin gelatin of 0.8 % provides better yogurt quality compared to the use with Arabic Gum and CMC (Carboxymethyl Cellulose).

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

6. REFERENCES

1 B. Gimenez., M.C. Gomez-Guillen, and P. Montero. Storage of dried fish skins on quality characteristics of extracted gelatin. *Journal of Food Hydrocolloids,* 19(6), 958–963 (2005)

2. P.Zhou., and J.M. Regenstein. Effects of alkaline and acid pretreatments on alaska pollock skin gelatin extraction. *Journal of Food Science*, 70(6), 392–396 (2005).

3. B. Gimenez., J.Turnay., M.A. Lizarbe., P.Montero, and M.C. Gormez-Guillen. Use of lactic acid for extraction of fish skin gelatin. *Food Hydrocolloids,* 19(6), 941–950 (2005)

4. I. Pezron., M. Djabourov, and J. Leblond. Conformation of gelatin chains in aqueous solutions: A light and small angle neutron scattering study. *Journal of Polymer,* 32(17), 3201–3210 (1991).

5. M.I. Said., S. Triatmojo., Y. Erwanto dan A. Fudholi. Karakteristik Gelatin Kulit Kambing yang Diproduksi melalui Proses Asam Basa. *Agritech,* 31(3), 0216–0455 (2011).

6. L.M. Kasankala., Y. Xue., Y. Weilong., S.D. Hong, and Q. He. Optimization of gelatine extraction from grass carp (Catenopharyngodon idella) fish skin by response surface methodology. *Bioresource Technology.* 98(17), 3338–3343. (2007).

7. H. Shuxian., L. Laihao., Y. Xianging., C. Jianwei., S. Honh., B. Qi, and J. He. The characteristics of gelatin extracted from sturgeon (Acipenser baeri) skin using various pretreatments. *Food Chemistry,*115(1), 124–128 (2008).

8. M. Ulfah. Pengaruh konsentrasi larutan asam asetat dan lama waktu perendaman terhadap sifat-sifat gelatin ceker ayam. *Agritech*,31(3), 161–167 (2011).

9. M.I. Said., S. Triatmojo., Y. Erwanto, and A. Fudholi. Gelatin Properties of Goat Skin Produced by Calcium Hydroxide as Curing Material. *Media Peternakan,* 34(3), 184–189 (2011).

10. D. Masilamani., B. Madhan, and G. Shanmugan. Extraction of collagen from raw trimming wastes of tannery: A waste to wealth approach. *Journal of Cleaner Production,* 113(1), 338–344 (2016).

11. K.S. Sasmitaloka., Miskiyah, dan Juniawati. Kajian Potensi Kulit Sapi Kering Sebagai Bahan Dasar Produksi Gelatin Halal. *Buletin Peternakan*, 41 (3), 328–337 (2017).

12. M. Sompie., S. Triatmojo., A. Pertiwiningrum., and Y. Pranoto. The Effects of Animal Age and Acetic Acid Concentration on Pig Skin Gelatin characteristics. *Journal of the Indonesian Tropical Animal Agricuture*, 37(3), 176–182 (2012).

13. T.J. Sims., A.J. Bailey, and D.S. Field. The Chemical Basis Of Molecular Weight Differences In Gelatins. *The Imaging Science Journal, 4*5(3), 171–177 (1997).

14. S. Wangtueai, and N. Athapol. Processing optimization and characterization of gelatin from lizardfish (Saurida spp.) scales. *Journal of Food Science and Technology,* 42(4), 825–834 (2009).

15. J.A. Arnesen, and A. Gilberg. Preparation and characterization of gelatine from the skin of harp seal (Phoca groendlandica). *Bioresource Technology,* 82(2), 191–194 (2002).

16. AOAC. Official Methods of Analyses Association (18th Ed), A*ssociation Analytical Chemist, Washington, D.C.* (2005).

17. A,N. Hassan., J. Frank., K. Schmidt., S. Shalabi. Textural properties of yogurt made with encapsulated nonropy lactic cultures. *Journal of Dairy Science ,* 79(12), 2098–2103 (1996).

18. A.Chinnaswamy., Reilly., A.G.F. Stapley. Effects of Process Variables on the Denaturation of Whey Proteins during Spray Drying. *Drying Tecnology,* 25(4-6), 799–807 (2007).

19. I. Kolodziejska., K. Kaczorowski., B. Piotrowsia, and M. Sadowska. Modification of properties of gelatin from skins of Baltic cod (Gadus morhua) with transglutaminase. *Food Chemistry.* 86(2), 203–209 (2004).

20. Mondal and P. K. Chattopadhyay. Mechanical and hydrodynamic swelling characteristics of bovine tanned leatherpost-treated by acrylic and glutaraldehyde tanning agents. *Bangladesh Journal*

of Scientific and Industrial Research, 51(1), 1–12 (2016).
21. C.H. Wang., F. Li., and N. Takhahasi. The Renin Angiotensin System and The Metabolic Syndrome. *Journal American Medical Association.* 100(5), 525–34 (2010).
22. A.G.Ward. The physical properties of gelatin solutions and gels. *British Journal of Applied Physics,* 5(3), 85–88 (2002).
23. T. Amatayakul., F. Sherkat, and N.P. Shah. Syneresis in Set Yogurt as Affected by EPS Starter Cultures and Levels of Solids. *International Journal of Dairy Technology,* 59(3), 216–221 (2006).
24. F. Zhang., Shiying Xu, and Z. Wang. Pre-treatment optimization and properties of gelatin from freshwater fish scales. *Journal Food and Bioproducts Processing,* 89(3), 185–193 (2011).
25. D.I. Zeugolis., S. T. Khew., E. S. Y. Yew., A. K. Ekaputra., Y. W. Tong., L. L. Yung., D. W. Hutmacher., C. Sheppard, and Michael. Electro-spinning of pure collagen nano-fibres – Just an expensive way to make gelatin. *Biomaterials,* 29(15), 2293–2305 (2008).
26. K.G. Harvinder. Properties of Gelatin from Skins of Fish Black Tilapia (Oreochromis mossambicus) and Red Tilapia (Oreochromis nilotica). *Jurnal Food Chemistry,* 77(1), 81–84 (2002).
27. R. Avena-Bustillos., C.W. Olsen, and D.A. Olson. Water Vapor Permeability of Mamalian and Fish Gelatin Films. *Journal of Food Science*, 71 (4), 202–207 (2006)
28. A.A. Karim, dan R. Bhat. Review Fish Gelatin. properties challenges and prospectes as an alternative to mammalian gelatins. *Food Hydrocolloids*, 23(3),563–576 (2009).
29. L.P. Burin, and P. Buera. B- Galactosidase activity of affected by apparent pH and physical properties of reduced moisture systems. *Enzyme and Microbial Technology*, 30(3) , 367–373 (2002).
30. S.S. Choi and J.M. Regenstein. Physicochemical and Sensory Characteristics of Fish Gelatin. *Journal of Food Science,* 65(2), 194–199 (2000).
31. H.V. Atherton, and J.A. Newlander. Chemistry and Testing Dairy Products. *The Avi Publishing Company Inc. Connecticut* (1981).
32. Committee on Food Chemicals Codex. Food Chemicals Codex. *National Academy Press. Washington, D.C.* (1996).
33. Steel R G D and Torrie J H . Principles and Procedures of Statistics 2nd Ed .Tokyo: *International Book Company.* 1991