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Review on Biochemical Composition and Microflora of Prawns

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Abstract

Prawns are highly demanded seafood commodity both in domestic and International markets due to their nutritional value and unique taste. However, its trading is a risky business due to quick perishable nature and the formation of black spots on the exoskeleton. Biochemical composition such as water activity, pH and autolytic enzymes of the prawn significantly influence the degree of spoilage; moreover, prawns are scavengers and filter feeders which cause them to accumulate several bacteria in their body, which also accelerates the spoilage process. Hence, basic information regarding the biochemical composition and micro-flora of prawn are highly relevant to find out suitable preservation techniques and the production of various value added products. This article reviews the available information on the moisture, sarcoplasmic protein, myofibrillar proteins, stroma protein, non-protein nitrogen (NPN), free amino acid, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids (PUFA), mineral content and microflora of the prawns.

Keywords: Moisture, protein, lipid, mineral, fatty acids, microflora, prawn

Introduction

Seafood is a good source of proteins, lipids, vitamins and minerals which are vital for growth, development and health. Among the seafood, prawns are considered to be a healthy choice as food because of the high quality protein and very low fat and calories. According to Adeyeye (1996), prawns are better source of animal protein due to its abundance and reasonable price. Dong (2001) suggested that nutrients of shellfish are highly necessary for the growth of human body. Prawns also contain significant quantities of vitamin A & D, and several dietary minerals such as calcium (Ca), iron (Fe), etc. which are beneficial to consumers. Iodine content in prawn is good for proper functioning of the thyroid gland. Biochemical composition, such as protein, amino acids, lipids, fatty acids, carbohydrates, vitamins, minerals, related biomolecules are highly influenced to the nutritive values of crustaceans. Thenutritive values of crustaceans depend upon their body biochemical constituents. Vijayavel & Balasubramanian (2006) found that biochemical composition of crustaceans greatly influence its nutritional quality. In global food markets prawns are commercially traded seafood item having a higher market value. Moreover, prawns significantly contribute towards export earnings of our country. On a value basis, prawn is the major export commodity compared to other seafood item exported from India. Overall, export of prawn during 2014-2015 was 3, 57, 505 metric ton (MT) worth 3709.76 million US $ which accounts for 78.7% of the total revenue generated through export (MPEDA, 2015). Commercial prawns are classified into penaeid and non-penaeid group. Important species of penaeid and non-penaeid prawns exploited from Indian water are *Fenneropenaeus indicus, Penaeus merguiensis, P. monodon, P. semisulcatus, Metapenaeus dobsonii, M. affinis, M. monocerus, M. brevicornis* and *Parapenaeopsis stylifera*.

Prawns are highly perishable due to water activity, pH and autolytic enzymes (Dalgaard, 2000). Prawns are demersal and filter feeders which cause them to accumulate several bacteria and viruses in their body. Significant quantities of free amino acid content in prawn muscles make them easily susceptible to bacterial spoilage (Simidu, 1962).

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Black spot formation in prawn reduces its value in the export market. In order to develop novel preservation techniques for the extension of shelf life of prawn, basic information on its biochemical composition and micro-flora are highly necessary.

Proximate composition of prawn

Proximate composition means relative amounts of water, protein, lipid, ash and carbohydrate in an organism. Protein, lipid and carbohydrate each contributes to the total energy content of an organism; while, water and ash only contribute to the mass. Comprehensive reviews on the biochemical composition of fishes and shellfish have been done by Love (1974). Biochemical composition of fish and shellfish from Indian water was reported by Mukundan & James (1978), Gopakumar et al. (1993), Natarajan & Sreenivasan (1961). Proximate compositions are slightly different in prawns and lobsters (Rosa & Nunes, 2003); moreover, it varies in different regions of the body of an organism. Proximate composition of seafood varies due to several factors such as seasonal, climatic, geographic, habitat, developmental stage, sexual maturation, etc. (Ravichandran et al., 2009).

Moisture

Water is the principal component of seafood; it varies from 28 to 90% by weight (Stanby, 1962). Water content in prawn contributes significantly towards functional properties and organoleptic qualities. It acts as a good reaction medium for enzymes and also helps to maintain the proper shape of the organism. Water in the muscle is tightly bound to the proteins by hydrogen bonding as well as some covalent bonds, such water is called bound water and the remaining water is termed as free water. Water and lipid content have an inverse relationship such that the sum of these approximates 80% (Stanby, 1962). Moisture content of 14.7% (on dry basis) was reported in the flesh of Indian white prawn by Ravichandran et al. (2009).

Protein

Proteins were first described by the Dutch chemist Gerardus Johannes Mulder and coined by the Swedish chemist Jöns Jacob Berzelius in 1838. Proteins are generally high molecular weight nitrogenous substances which are colourless, amorphous and colloidal in nature. Prawn proteins are easily digestible and nutritionally superior because of the availability of essential amino acids. Prawn meat is an excellent source of protein (Yanar & Celik, 2006). Generally, shellfish contains 8-15% of protein and in fish it ranges from 15-24%. Prawn muscle contains hundreds of different proteins along with other non-protein nitrogen compounds. Muscle proteins together with non-protein nitrogen are referred to as crude protein. Haard (1995) reported higher crude protein content in crustaceans than molluscs. Higher protein content in juvenile prawns was due to the accelerated protein synthesis at active growth phase stage (Pedrazzoli et al., 1998). Crude protein content of Indian white prawn was reported as 20.90% (Gopakumar et al., 1993) and a similar result was reported by Joseph et al. (1998). Konosu et al. (1958) found that marine prawn can have up to 23% of crude protein. Ravichandran et al. (2009) reported that the percentage of protein (on dry basis) in the flesh of Indian white prawn was 41.3%. Gomezet al. (1988) and Habashy (2009) suggested that diet composition of prawn significantly alters the protein content of prawns. Protein content of prawn varies with several factors such as nutrition, stage of the reproductive cycle and specific properties of different parts of the organism (Sikorski & Kolakowska, 1994). Generally, proteins are classified into three types based on their solubility that are sarcoplasmic, myofibrillar and stroma protein.

Sarcoplasmic protein

Sarcoplasmic proteins in seafood are more or less similar to land animals and include myoglobin, hundreds of enzymes and other albumins (Haard et al., 1994). These enzymes are highly important because they influence the edible qualities of seafood (Simpson et al., 1991). Haard (1990) reviewed the comparative aspects of muscle enzymes in seafood. Importance of enzymes present in the sarcoplasm of fish and shell fish has been reviewed by Simpson et al. (1991).

Myofibrillar proteins

Myosin and actin are the myofibrillar proteins that directly function in the contraction-relaxation cycle (Haard, 1995). Other proteins that participate indirectly in the cycle are called regulatory proteins (Maruyama & Ebashi, 1970) which include troponin, tropomyosin and actin. Concise and informative reviews on myofibrilar proteins have been published by Maruyama (1985), Squire& Vibert (1987), Morrissey et al. (1987), Nakai & Li-chan (1988) and

Sikorski (1994). Myosin heavy chain is the major protein in myofibrillar protein (Shahidi, 1994). Proteins and their structure in fishes and shellfish were reviewed by Olley (1980) and Sikorski (1980).

Stroma protein

Stroma protein is composed of collagen and elastin from connective tissues (Sikorski & Borderias, 1994). Collagen is the dominant stromal protein in the prawn meat (Foegeding et al., 1986). Collagen plays an important role in the maintenance of texture in an organism. Yoshinaka et al. (1989) has reported the presence of collagen in the musculature of several crustaceans. Texture of the prawn meat is highly influenced by collagen content (Sato et al., 1986). Yoshinaka et al. (1989) reported the similarity of major collagen in the crustacean muscle and type V collagen in vertebrate muscle. Crustacean collagen has a high biological value due to the presence of several essential amino acids (Sikorski et al., 1984; Ashie & Simpson, 1997). However, degradation of stromal protein causes mushiness in freshwater prawn (Nip et al., 1985). Crawford (1979) revealed that prawn collagen was more susceptible to hydrolysis by proteinase than fish collagen. Degradation of this collagenous matter has a great role in the textural changes of seafood during chilled storage.

Non-protein nitrogen (NPN)

Non-protein nitrogen fraction includes ammonia, peptides, amino acids, amines, amine oxides, guanidine compounds, quaternary compounds, purine and urea which are mostly found in the sarcoplasm of the muscle. Non-protein nitrogen contributes significantly to the unique taste of seafood as well as to its spoilage (Simidu, 1962; Haard, 1992). Black tiger prawn shows more non-protein nitrogenous components than white shrimp (Sriket et al., 2007). About 95% of the total amount of NPN available in marine finfish and shellfish muscle is composed of free amino acids, imidazole, dipeptides, TMAO (trimethylamine oxide) and its degradation products like urea, guanine compounds, nucleotides, betaines, and products of post-mortem changes (Ikeda, 1980). Peptides are a group of non-protein compounds that contribute around 15% of the total NPN in crustaceans (Finne, 1992). Betaines are another major NPN which might be present up to 10% of the total NPN. Konosu & Hayashi (1975) reported that marine invertebrates like crustaceans contain higher level of betaines. Glycine betaine is one of the naturally occurring betaines in shellfish generally seen in higher concentration (Beers, 1967). â alanine betaine and carnitine are other betaine which are frequently found in various invertebrates (Hayashi & Konosu, 1977). Higher level of nucleotide has been reported in squid and prawn than in other invertebrates (Arai, 1966). Mendes et al. (2001) reported AMP and ATP were the dominating nucleotides in crustaceans and molluscs. However, creatine and creatine phosphate were reported to be higher in vertebrates (Watts & Watts, 1974) than invertebrates (Mathew et al., 1999). Suryanarayana et al. (1969) reported the accumulation of inosine monophosphate (IMP) in prawn. Sikorski et al. (1990) suggested that the nucleotides and quaternary ammonium compounds have a great role in the taste of seafoods. Olley & Thrower (1977) reviewed nucleotide and other chemical components in marine invertebrates. Mathew et al. (1999) made an attempt to understand the distribution of major NPN constituents in the muscle of fish and shellfish. Sikorski (1994) found that the level of NPN content in marine animals depends on the species, habitat, life cycle and state of freshness after the catch. NPN compounds of fish and shellfish were reviewed by Simidu (1962), Ikeda (1980) and Finne (1992).

Free amino acid

Amino acids are building blocks of proteins. Free amino acid content in the muscle influences the characteristic flavor of fish and shellfish (Simudu et al., 1953; Hashimoto, 1965; James, 1969) and plays a great role for osmoregulation in crustaceans (Bedford & Leader, 1977; Finne, 1992). McCoid et al. (1984) found that salinity affects the composition and concentration of free amino acids in *Penaeid* prawn. Bailey (1937) made an attempt to understand the amino acid composition of myosin in fish and shellfish. Some studies have been conducted to determine an amino acid profile of *Parapenaeus* sp (Dabrowski et al., 1969) and *P. monodon* (Yaquan et al., 1995). Little variation of amino acid profile in different species of prawn was found by Anon (1959). James (1969) estimated free amino acid composition of different species of prawn from India and Haard (1992) found that free amino acid content in the muscle of aquatic organisms ranges from 0.5 to 2% by weight. Yanar & Celik (2006) reported different amino acid compositions in green tiger shrimp (*Penaeus semisulcatus* De Haan, 1844) and speckled shrimp (*Metapenaeus monoceros* Fabricus, 1789) and the authors also reported arginine, proline, leucine, isoleucine, phenylalanine and glutamic acid were abundant in both species. Konosu (1971) revealed that free amino acids are rich in crustaceans and molluscs whose nitrogen content accounts for 20-70% of the total NPN. In invertebrates, free amino acid nitrogen contributes 40% of the total NPN (Velankar & Govindan, 1958) and in prawns it was 65% (Finne, 1992). Simidu & Hujita (1954) reported 80-160 mg of glycine nitrogen 100 g^{-1} in prawn meat. Other than glycine, prawn muscle is also rich in proline, arginine, serine, threonine and alanine (McCoid et al., 1984). Sikorski et al. (1990) suggested that high content of free arginine provides the sweet taste and seafood-like flavour to the crustaceans; whereas arginine, leucine, valine, methionine, phenylalanine, histidine and isoleucine give bitter taste. Alanine, proline and serine contribute to the acceptability of prawns and lobsters (Fuke, 1994).Thompson &Thompson (1968) reported that tryptophan residues partially replace the residues of amino acids. Sikorski (1994) compiled the information regarding the amino acid composition of actin and troponin isolated from different invertebrates and fishes.

Lipids

Lipid may either occur as triacyl glycerols, the main form in which energy resources are stored or as mostly phospholipids and cholesterol that form the essential component of cellular and sub cellular structures (Love, 1997). Phospholipids were the major lipid component (72–74%) followed by triglyceride in black tiger prawn and white shrimp (Sriket et al., 2007). Yepiz-Plascencia et al. (2000) also reported that phospholipids are the predominant lipid in crustaceans. Lopez & Maragoni (2000) reported that lipase and phospholipase in prawn meat accelerate the hydrolysis of lipid. Sriketet al. (2007)found that lipid content in white shrimp are highly susceptible for hydrolysis by lipase or phospholipase than black tiger shrimp due to the presence of higher free fatty acid and lower diglyceride content. Information on lipid content of many fish and shellfish from India has been reported by Gopakumar et al. (1993), Gopakumar & Rajendranathan (1972) and Nair & Gopakumar (1978). Crude lipid content (on wet basis) of Indian white prawn was 7.6 and 9.8% in shell and muscle, respectively (Ravichandran et al., 2009). According to Sriket et al. (2007) aquaculture prawns have higher crude fat content than wild captured prawns. Total available lipid in shellfish usually ranges from 1 to 2% (King et al., 1990). Edible parts of marine prawn showed 1-2% of lipid (Johnston et al., 1988), which is agreement with Karapanagiolidis et al. (2010). Lipid content of seafood varies with species, season, physiological condition and spawning, locations in the muscle or organ, between wild and cultivated sources, diet of the fish and geographic locations, etc. (Jacquot, 1961).

Marine lipid is generally characterized by their great variety of saturated and unsaturated fatty acids which are commonly associated with a complex mixture of triglycerides (Sinnhuber, 1968). Marine lipids contain 50-60 numbers of natural fatty acids (Ackman, 1974) and among these only 14 fatty acids are important in terms of weight percent composition (Ackman & Eaton, 1971). Fatty acids of marine lipids were reviewed by Ackman (1974; 1980; 1995) and Krzynowek & Murphy (1987). Information on fatty acid composition of fish and shellfish from India was discussed by Gopakumar et al. (1993), Gopakumar & Rajendranathan (1972) and Nair & Gopakumar (1978).

Saturated fatty acids

Saturated fatty acids usually constitute 20-35% of the total fatty acids in fish and shellfish (Ackman, 1995). As in most animals, palmitic acid (16:0) is the principal saturated fatty acid that constitutes about 10-30% of the total fatty acid with some degree of inter-changeability with myristic acid (14:0) whose contribution varies between 5-10% (Ackman, 1974). Hamid et al. (1978) and Kunimoto et al. (1975) were of the view that most of the *iso* and *anti-iso* odd chain fatty acids contribute to the lipid of the aquatic food chain.

Monounsaturated fatty acids

Marine lipids contain four important naturally occurring mono ethylenic fatty acids like C 16:1, C 18 :1, C 20:1 and C 22:1. Among these, C 16:1 and C 18 :1 contribute to about 5-15% and 10-30% of the total lipid in that order of depot triglyceride fat and less than 5% in phospholipids (Ackman, 1974). Lambertsen (1972) reported that C 20:1 and C 22:1 fatty acids are more significant in depot fat than in phospholipids where they are present only in modest amounts. According to Ackman (1974), minimum content of C 20:1 and C 22:1 acids are essential for fatty fishes. Occurrence of number of isomers of monoethylenic fatty acids in fish lipid was reviewed by Ackman (1980).

Polyunsaturated fatty acids (PUFA)

Most important fatty acids found in fish lipids are 18:2 n-6, 18:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3 (Ackman, 1995). The presence of one to six double bonds in fatty acids gives marine oil their most specific characteristics (Gauglitz et al., 1974). Feliz et al. (2002) suggested that prawn meat is an excellent source of poly unsaturated fatty acids (PUFA) such as eicosapentaenoic (20:5n3, EPA) and docosahexaenoic (22:6n3, DHA) acids. Higher PUFA content in commercially important prawns like *Peaneus monodon*, *P. indicus, P. semisulcatus, Litopenaeus vannamei, Metapenaeus monoceros* and *M. dobsoni* were reported by Shahina et al. (2016). Lin et al. (2003) reported the major fatty acids in white shrimp were PUFA such as C22:6 n " 3(DHA) and 20:5 n – 3(EPA). Rosa & Nunes (2003) and Yanar & Celik (2005) found higher content of palmitic acid (C16:0), stearic acid (C18:0), DHA and EPA in *Nephrops norvegicus*, *Parapenaeus longirostris*, *Aristeus antennatus, P. semisulcatus* and *M. monoceros*. Ackman (1980) reported that linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) do not accumulate in fish and marine invertebrates to the extent of more than 1-2% of fatty acid. Ackman (1995) suggested that eicosapentaenoic acid (EPA) (20:5 n-3) and decosahexaenoic acid (DHA) (22:6 n-3) are the dominant PUFA's in seafood. The beneficial effect of PUFA of ù-3 family, particularly EPA and DHA in seafood was reviewed by Kremer et al. (1987) and Benitez (1989). In crustaceans, 55-90% of the total fatty acids consist of 16:0, 16:1 ω7, 18:1 ω9, 20:5 ω3 and 22:6 ω3 fatty acids (Karakoltsidis et al., 1995). Johnston et al. (1988) revealed that ω-6 fatty acids were the major fatty acid in fresh water prawn; whereas, in marine prawn, it was ω-3 fatty acids. However, fatty acid composition and cholesterol content of prawn vary with seasonal changes (Luzia et al., 2003).

Minerals

Aquatic organisms absorb minerals from their diet and surrounding water which gets deposited in skeletal tissue, muscle and different organs (Lall, 1989). Minerals play a vital role in the formation of skeletal structure, maintenance of colloidal system and regulation of acid base equilibrium besides being a key component of hormones, enzymes and enzyme activation (Lall, 1989). Generally, invertebrates are rich in minerals (Yanar et al., 2006). Mineral constituents of fish and shellfish were reviewed by Causeret (1962) and the information was compiled by Sidwell et al. (1974) and Teeny et al. (1984). Gopakumar et al. (1993) have reported the mineral content of fish and shellfish. Abulude et al. (2006) reported significant content of minerals such as Ca, P, Mg, Mn and Cl and Vitamins (A, C, and D) in prawns. Yanar et al. (2006) found that Ca, K, P, Na and Fe are the major minerals in *P. semisulcatus* and *M. monoceros*. Transition metal like Cu was found in haemocyanin of crustaceans (Decker & Tuczek, 2000). Lall (1989) reported that crustaceans contain more calcium than finfish because of its requirement of calcium for shell formation and muscle function. Dyer et al. (1977) and Teeny et al. (1984) recorded lower level of potassium in shellfish. Hemocyanin, the blood pigment of crustaceans contains copper (Decker & Tuczek, 2000). Most of the shellfish have sodium in the range of 120-440 mg 100 g^{-1} except for the squid and scallop (Lall, 1989).

Ravichandran et al. (2009) revealed mineral constituents of Indian white prawn. The authors reported the mineral content such as sodium in the flesh and shell of the *F. indicus* were 29 mg 100 g^{-1} and 38.6 mg 100 g^{-1}, respectively; whereas, the potassium content in flesh was 24.3 mg 100 g^{-1} and in shell was 33.2 mg 100 g^{-1}; similarly, better phosphorus content of 82.4 mg 100 g^{-1} to 91.5 mg 100 g^{-1} and calcium and magnesium was observed in prawns. Prawn meat is an excellent source of calcium (Yanar & Celik, 2006). Yanar et al. (2006) observed that the mineral content of *P. semisulcatus* and *M. monoceros* vary with season except for Ca content in *P. semisulcatus.* Ravichandran et al. (2009) reported the mineral content such as sodium, potassium, phosphorus, calcium and magnesium in Indian white prawn meat was in the range of 29 mg 100 g^{-1}, 24.3 mg 100 g^{-1}, 82.4 mg 100 g^{-1} and 91.5 mg 100 g^{-1}, respectively. Yanar et al. (2006) observed that the mineral content of *P. semisulcatus* and *M. monoceros* vary with season except for Ca content in *P. semisulcatus*.

Microflora in the prawns

Microflora in freshwater and marine crustaceans reflect the microbial load in the ecosystem (Liston, 1980). Cann (1977) published major groups of bacterial flora in shellfish which were *Micrococcus*, *Coryneforms, Moraxella/Acinetobacter, Pseudomonas,* and to a lesser extent *Flavobacterium/Cytophaga and Bacillus*. It was also reported that the microflora of prawn contains mostly Gram positive bacteria belonging to the *Coryneform* group or *Micrococcus.* A high proportion of *Coryneform* bacteria in crustaceans from temperate waters were reported by Hobbs et al. (1971). Contrary to Cann (1977), Karthiyani & Iyer (1975) reported Gram negative flora comprising of the genera *Pseudomonas, Achromobacter* and *Vibrio* were predominant in commercially important prawns and lobsters. Surendran (1980) found that around 86% of the total flora in *P. indicus* comprises of Gram negative asporogeneous rods and cocci. In another study, Surendran et al. (1985) observed that the initial bacterial load on Indian white prawndominates Gram negative genus which were *Pseudomonas* (12%), *Acinetobacter* (24%), *Moraxella* (28 %), *Vibrio* (5%), *Flavobacterium/Cytophaga* (6%) and *Arthrobacter* (12%). Chandrasekaran et al. (1985) found *Vibrio* sp. are the major flora in the spoiled prawn. *Aeromonas, Pseudomonas, Vibrio, Flavobacterium and Serratia* were the major bacteria flora reported in fresh white prawn in which *Aeromonas* constitutes about 38% of the total flora (Jeyasekaran et al., 2006). *Micrococcus, Aeromonas* and *Moraxella* were the predominant microflora in ice-stored *F. indicus* (Jeyaweera & Subasinghe, 1988). Microflora of farm reared ice-stored *M. rosenbergii* comprises of *Pseudomonas, Aeromonas hydrophila, A. veronii boivar* and *Shewanella putrefaciens* (Lalitha & Surendran, 2006). *Escherichia coli* is aerobic, Gram negative, highly motile, non-spore forming and rod shaped bacteria. Host of the bacteria is the intestinal tract of man and warm blooded animals (Huss, 1994) and its presence in food indicates contamination with faeces of human and animal origin (Bej et al., 1990). Therefore *E. coli* is also known as faecal coliforms (Paul et al., 1960). *E. coli* causes infection in humans and animals resulting in a wide variety of intestinal diseases (Salyers et al., 1994). Varma et al. (1985) reported that *E. coli* is commonly found in frozen prawn and cuttle fish. Earlier works reported the occurrence of *E. coli* in freshly caught marine prawns (Jayaweera & Subasinghe, 1990). Sahu et al. (1998) isolated *E. coli* including enterotoxigenic *E. coli* sero type from prawn. Limit of *E. coli* in raw fish and fishery products is 20 g^{-1} and it should be absent in cooked fishery products. Pathogenic bacteria causing disease to prawns were reviewed by Lavilla-Pitogo, (1995) and Karunasagar & Karunasagar, (1996).

Conclusion

Significant quantities of moisture, protein, lipid and mineral content were reported in prawns. Presence of poly unsaturated fatty acids such as µ-6 fatty acid in fresh water prawn and µ-3 fatty acids in marine prawn were also reviewed. Vitamin A & D and several dietary minerals such as calcium (Ca) and iron (Fe) that occurs in prawns also give the health benefits to the consumer. Similarly, non-protein nitrogen (NPN) fraction such as ammonia, peptides, amino acids, amines, amine oxides, guanidine compounds, quaternary compounds, purine and urea contribute unique taste to prawns. Free amino acid content in the muscle influences the characteristic flavour of shellfish and plays a great role for osmoregulation. Gram negative bacteria such as pseudomonas, acinetobacter, moraxella, vibrio, flavobacterium/cytophaga and arthrobacter were the major bacterial flora in prawns. However, these parameters vary with several factors such as species, habitat, life cycle and specific properties of different parts of the organism and state of freshness after the catch.

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