Ageing related changes in quantity and quality of saliva: Where do we stand in our understanding?

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ABSTRACT

Saliva is crucial to oral processing of food and consequently is also related to the sensory and textural experience. It is often assumed that the secretion and properties of saliva change with age, which can result in dry mouth conditions, taste aberrations. Such changes may result in reduced nutrient intake and malnutrition besides adversely affecting the quality of life. Based on some recent research findings, this paper reviews our current understandings on age-dependent changes on quantity (bulk salivary flow rate) as well as quality of saliva (e.g. composition, viscosity, lubrication) in healthy elderly individuals. The review begins with a short introduction to histological changes of salivary glands upon ageing. This is followed by covering different aspects of salivary changes with key articles highlighting decreased flow rate, increased ionic concentration, decreased calcium and mucin content in saliva of elderly subjects consequently affecting the oral coating and flavour perception. We also highlight issues in data associated with respect to variance in saliva collection protocols as well as factors influencing such results other than age, such as health conditions and polypharmacy. Clear gaps in literature have been highlighted with respect to lack of quantitative data in viscoelasticity, rheology and lubrication properties of saliva in healthy elderly population and the potential impact of changes in these material properties on sensory and textural perception of food and consequently food intake. Such insights will not only have clinical implications for maintaining optimal oral health in elderly population but also serve to optimize food for elderly population.

KEYWORDS (5): Ageing; saliva; dry mouth; rheology; tribology

PRACTICAL APPLICATIONS

The population has undergone a fundamental change in its age structure globally, with a rapid increase in elderly population. Innovation of tailored foods is still in its early stage to satisfy the needs of growing ageing population. One of the biggest challenges in such food product development is lack of adequate understanding and characterisation of endogenous factors i.e. age-related changes in saliva, which may influence oral processing of food and subsequently nutrient intake. Ageing affects the salivary glands and alters quantity (flow rate) and quality (e.g. ion and protein composition, rheology, tribology) of saliva. Thus, older adults may suffer from dry mouth, taste aberration, and poor oral hygiene, greatly affecting their quality of life. This review provides insights into how age versus other health conditions influence salivary properties. Understanding of age-dependent changes in salivary rheology and tribology will be of paramount importance to optimize food for elderly population.

1. INTRODUCTION

Population ageing is occurring globally at an unprecedented rate with the proportion of people aged over 60 years being estimated to double by 2050 (Lancet, 2014). Besides affecting physical and physiological capabilities, ageing significantly impacts nutritional status by other means. For instance, defective teeth or teeth loss, poor oral hygiene, lack of saliva resulting in impaired oral processing, taste and texture aberrations can result in reduced nutrient intake and consequently malnutrition (Coleman, 2002; Laguna et al., 2015; Laguna et al., 2016a; Laguna et al., 2016b; Mingioni et al., 2016; Laguna et al., 2017c). Swallowing disorders (dysphagia) and dry mouth (xerostomia, salivary gland hypofunction) are the most prevalent oral processing conditions encountered by ageing adults. Xerostomia is a subjective sensation related to mucosal dehydration and reduced oral lubrication, which is not necessarily linked to salivary gland hypofunction (Navazesh et al., 1992; Pajukoski et al., 2001; Nagler and Hershkovich, 2005; Liu et al., 2012; Villa et al., 2015). In patients suffering from Xerostomia, secreted saliva lacks the ability to continuously wet the whole oral cavity uniformly (Närhi, 1994). People suffering from dry mouth conditions will not only have problems of food mastication and swallowing, but also encounter problems of taste, speech and reduced tolerance of dentures. Furthermore, xerostomia increases the risk of dental caries, periodontal disease, candidiasis, oral ulceration, dysphagia, all of which can adversely impact nutritional status and quality of life (Mandel, 1989; Vissink et al., 1996).

Xerostomia has many causes, including autoimmune exocrinopathy (e.g. primary Sjögren's syndrome), prescribed medication/polypharmacy, radiotherapy, sarcoidosis, HIV and poorly-controlled diabetes mellitus (Mortazavi et al., 2014). Renewed research interest in dry mouth has been driven by the increase in elderly population with chronic diseases and polypharmacy that in turn commonly induce mouth dryness (Ship et al., 2002; Guggenheimer and Moore, 2003). Ageing affects the ability to taste and smell due to diminished cognition, salivary hypofunction and diminished chewing ability due to the loss of dentures. All of these may lead to changes in the regulation of appetite provoking a lack of hunger, also known as "anorexia of ageing" (Malafarina et al., 2013).

It is often believed that xerostomia in elderly is ultimately associated with age-dependent changes in quantity and quality of salivary secretion (Vissink et al., 1996). Saliva is a complex biological fluid naturally secreted inside human mouth that is essential for eating to form a coherent, smooth and swallowable bolus (Prinz and Lucas, 1997). Saliva also plays an important role in sensory perception by diluting food components responsible for taste and aroma, allowing them to interact with the taste buds (Doyennette et al., 2011; Neyraud, 2014). In addition to the eating-related functions, saliva secretion ensures continuous hydration of the mouth and demonstrates an antibacterial function (Dowd, 1999).

From a compositional viewpoint, saliva is a slightly acidic fluid mixture mainly composed of water (99.5%), proteins (0.3%), including mucins and enzymes and inorganic substances (0.2%) (Humphrey and Williamson, 2001). Saliva also adheres to oral surfaces and helps to maintain saliva pellicle thickness of 30-100 nm (Lendenmann et al., 2000; Morzel et al., 2014; Hannig et al., 2017) although the thickness may vary depending upon the pellicle's location in the mouth. Proteins, such as anionic glycosylated mucins, statherins render saliva with its rheological (viscosity, elasticity, stickiness), unique water-holding and lubrication properties (Douglas et al., 1991; Sarkar and Singh, 2012; Sarkar et al., 2016; Laguna and Sarkar, 2017).

To perform salivary studies, researchers collect the saliva by stimulating saliva production or without stimulation (unstimulated saliva). Saliva is allowed to accumulate in the floor of the mouth and the participants spit into a pre-weighed cup for a period (usually 5 min). This is referred to as 'unstimulated saliva' where salivary flow is not influenced by external stimuli. 'Stimulated saliva' collection also uses a pre-weighed cup, but the participants actively spit using a chemical or mechanical stimulant in advance, such as citric acid solution, or chew a piece of paraffin-wax, gauze or other inert chewable materials (Smith et al., 2013). Unstimulated saliva is mainly secreted from the sublingual and submandibular glands, while stimulated saliva is secreted mostly by the parotid gland. Stimulated saliva contains lower quantities of protein (e.g. glycosylated mucin) and has a lower viscosity than that of the unstimulated counterpart. Many studies demonstrated differences in rheological and tribological properties between these two types of saliva (Prinz et al., 2007; Silletti et al., 2008).

Unstimulated salivary flow rate is the most commonly employed measure for the quantity of saliva (Navazesh et al., 1992). The rate of saliva secretion varies hugely among individuals, depending on an individual's health status and physiological conditions. The average saliva secretion rate ranges from 0.5 to 1.5 L/per day, showing its dependence on circadian rhythms (Pedersen et al., 2002). For quality of saliva, researchers use different measures, such as material properties (e.g. viscosity, coefficient of friction) and/or chemical analysis (e.g. mucin, statherin concentration, degree of glycosylation) (Davies et al., 2014; Chaudhury et al., 2015). Although there have been excellent reviews covering the physiological changes of saliva during ageing (Vissink et al., 1996; Nagler, 2004), it is important for food scientists to have a thorough understanding of the qualitative and quantitative changes in properties of this complex fluid upon ageing. Such information is crucial to serve as a basis to optimize food design for the elderly population as the perception of the food may be driven significantly by the alteration of endogenous salivary properties rather than the exogenous food properties.

Hence, the aim of this review is to understand the changes in saliva induced by age. Firstly, we will briefly discuss the physiological changes in the salivary glands upon ageing with respect to histology. Then, we will focus on the ageing-related changes in quantity of bulk saliva with respect to flow rate. We will cover the quality of saliva with respect to compositional changes, particularly with respect to proteins and ions, which might impact the material properties (e.g. rheology, lubrication). Finally, we will highlight how such changes can impact the sensation of taste, smell and aroma. We conclude with a discussion on the future studies that need to be conducted to deepen the comprehension of salivary changes with ageing versus health conditions so that generated insights can serve to design targeted food and/or new oral dryness therapies.

2. AGEING-RELATED PHYSIOLOGICAL CHANGES IN SALIVARY GLANDS

In humans, there are three major pairs of salivary glands that secrete 92-95% of the secreted saliva (parotid, submandibular and sublingual), whereas minor salivary glands are in the buccal, labial, palatal and lingual regions, including the base of the tongue that secretes the remainder (Paula et al., 2017). As shown in Figure 1, the parotid is the largest gland, situated in front of the ear and behind the lower jaw; submandibular glands are located in the posterior part of the floor of the mouth closure and are the smallest major glands. Sublingual glands are located in the floor of the mouth. Salivary glands are predominantly composed of three major types of cells (acinar, ductal and myoepithelial) (Figure 1), which contribute to salivary secretion into a series of small ducts opening under the tongue (Varga, 2015).

[Figure 1 here]

Saliva is primarily produced in the acinar cells, which also determine the type of secretion in the different glands; parotid gland: serous secretions; minor glands: mucous secretion; and sublingual and submandibular gland: mixed serous and mucous secretion (Mandel, 1987).

As it can be observed in the histological samples of Figure 2, the glands of a young individual 23 years old (y.o.) (Figure 2a), showed a more even and compact lobar structure with uniform appearance of parenchymal elements in comparison with an older individual (83 y.o.) (Figure 2b). With age, salivary gland histological studies have shown that although the number of ducts in the salivary glands remained the same, the proportional volume of fat and fibrovascular tissue increased in the parotid and submandibular glands in elderly individuals (Scott et al., 1987). On the other hand, the proportional volume of acinar cell secretion was reduced in elderly individuals (Vissink et al., 1996), being considered as one of the major causes of dry mouth (Vissink et al., 2010). All of these histological changes can result in overall salivary gland hypofunction (Vissink et al., 1996).

[Figure 2 here]

Regarding the minor glands, Sørensen et al. (2014) studied the labial salivary gland tissue from lower lip in 190 men (61 y.o.). About 33% of the participants displayed moderate to severe acinar atrophy and fibrosis (31%). Although xerostomia was not significantly correlated with histology alterations of labial salivary glands, it was inversely related to the total nerve length in the glandular connective tissue. Besides the histological gland atrophy, there are other causes reported for salivary gland hypofunction in elderly population. The physiological effect of ageing leads to changes in the salivary gland function due to a diminished intensity of the stimulation and reflex. With age, there is a reduction in the number of olfactory and taste receptors, diminished neuronal saliva stimulation (less transmitters acting on the receptors) and a decrease in the blood perfusion at the gland level. Furthermore, increase in ageing-related diseases and polypharmacy might also affect the gland function (Ekström et al., 2017).

3. CHANGES IN QUANTITY AND QUALITY OF BULK SALIVA ON AGEING

Changes in saliva on ageing can be grouped into quantitative (flow rate) and qualitative (composition, rheology, lubrication) properties.

3.1 SALIVARY FLOW RATES

Several studies have considered the changes in salivary flow rates with age. Interestingly, to our knowledge, no consensus has been reached on the decrease in salivary flow rate with age. This is largely attributed to the variations in the study design or saliva collection method. Affoo et al. (2015) performed a meta-analysis including all the published work regarding saliva and age. From the 47 studies finally selected, authors categorized the studies involving salivary flow rate in three the groups, 1) submandibular and sublingual saliva; 2) parotid gland and 3) minor gland salivary flow rate. Affoo et al. (2015) reported that both, unstimulated and stimulated average saliva flow rates were significantly lower ($p < 0.001$) in older adults than younger, with the difference being 66% higher in stimulated than in unstimulated saliva. Such decrease in salivary flow rate was specifically attributed to saliva from submandibular and sublingual salivary glands. However, parotid and minor gland salivary flow rates did not appear to be significantly lower.

Interestingly, polypharmacy, i.e. use of multiple medications, such as antidepressants, diuretics, analgesics, antihistamines, antihypertensives, antianxiety drugs, and appetite suppressant, which are routinely administered in the elderly population could not fully explain the differences in salivary flow rates between younger and older adults. A recent study further confirmed this by comparing healthy elderly (70-92 y.o.) and young subjects (22-55 y.o.) (Vandenberghe-Descamps et al., 2016). Researchers observed an average reduction of 38.5% in resting salivary flow rate and 38.0% of stimulated salivary flow rate in elderly subjects as compared to young subjects. The cause of salivary flow decline has been justified in the studies by age evolution of the salivary glands, as explained in the previous section: loss of acinar cells, loss of secretory tissue and adiposity increase as well as neurophysiological deterioration. Such decrease in salivary flow rate has an indirect influence of the quality of saliva, which is discussed in the next sub-section.

3.2 SALIVARY COMPOSITION

Most of the studies conducted with salivary changes are conducted with patients with burning mouth syndrome (Nagler, 2004), Sjögren's Syndrome (Chaudhury et al., 2016), or salivary analysis have been conducted after radiotherapy (Eliasson et al., 2005). Interestingly, studies on changes in salivary composition in healthy elderly individuals are relatively scarce. Table

1 summarizes the available studies with regards to changes in salivary composition in heathy elderly individuals in comparison with young individuals.

[Table 1 here]

As it can be observed, most of the components were studied by Nagler and Hershkovich (2005), where the authors found a significant increase in concentration of inorganic components in elderly (n=25, 70-86 y.o.) as compared to that of young population (n=26, 20-29 y.o.). Authors attributed this increase in ionic concentration of saliva to reduced salivary volume. As the water secretion pathway was affected with less salivary flowrate, there was a subsequent concentration effects on the ions. However, more recently, Nassar et al. (2014) found a decrease in calcium (Ca^{2+}) (Table 1) when comparing the two age groups of population (old: n=20, 60-80 y.o.; young: n=20, 30-60 y.o.) in the case of unstimulated saliva, which was not in agreement with the data reported by Nagler and Hershkovich (2005). Although the mechanism for salivary calcium decrease is unclear, such reduction of calcium has been previously observed in serum of healthy elderly subjects (Barbagallo et al., 1999). Furthemore, it should be noted that there is no standardised age category in the aforementioned studies, resulting in a potential source of variability in defining the elderly population.

For organic components, Nagler and Hershkovich (2005) found differences when reported as concentration versus total amount secreted (output). For instance, although the concentration of salivary proteins increased with age, this was not significant. However, when presented as output of secretion, salivary proteins decreased significantly ($p < 0.05$). For amylase, this was opposite i.e. the concentration was significantly higher (Table 1) but the output increase was not significant. This suggests that in some cases there is an age-dependent influence on secretion of one specific component, whereas, in others, the concentration effect is mainly driven by the decreased salivary output. Interestingly, there was a consensus particularly with respect to mucin concentration decrease with aging (Table 1). The salivary mucins (a group of different glycoproteins in saliva that covers the oral mucosa), form an immobile pellicle retained on epithelial cells (membrane-associated mucins: MUC1, MUC3, MUC4, MUC12) and a mobile salivary film (secreted soluble mucins: MUC2, MUC5A, MUC5B, MUC6, MUC7) (Cárdenas et al., 2007; Macakova et al., 2010; Morzel et al., 2014; Laguna and Sarkar, 2017). The MUC1 was reported to decrease in the elderly subjects; increasing the development of oral mucosal diseases in the aged population (Chang et al., 2011). In addition, Denny et al. (1991) and Navazesh et al. (1992) found that both levels of MUC1 and MUC2 in unstimulated saliva were significantly lower in the healthy aged group (65-83 years old) as compared to young adults (18-35 years old) (Table 1). With regards to general clinical knowledge, salivary components are important in maintaining oral health. Salivary proteins, such as mucin, lactoferrin, lysozyme, peroxidase) are required for non-immunological bacteria-defence system (Wu et al., 1994; Proctor, 2016). Various researchers observed reduced lactoferrin and peroxidase activity in the healthy elderly subjects, thus the modification in the balance between salivary antimicrobial agents contribute the impairment of oral tissues (Salvolini et al., 2000; Nagler, 2004; Dodds et al., 2005).

These findings agree with age-related histological and physiological changes in the salivary glands as previously discussed. Not only the quantity, but also the properties of mucins may influence dryness perception with the latter being associated with the changes in residual salivary composition and ions (Chaudhury et al., 2015). Dry mouth patients exhibit altered saliva rheological properties and reduced mucosal hydration, indicating functionally impaired saliva, largely linked to reduction in MUC5B and MUC7, as indicated in the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Figure 3).

[Figure 3 here]

Reduced amount of mucin results in a thinner adsorbed layer on the anterior hard palate i.e. thickness in the normosalivator is in the range of 7.6-57.2 μm, whereas in the case of hyposalivation, it is in the range of 3.4-25.7 μm. This is known as “enamel pellicle”, which induces weak oral mucosa protection and enamel demineralization (Lee et al., 2002; Lindh et al., 2014; Proctor, 2016). With reference to enamel demineralization, salivary calcium is required in reformation of enamel pellicle with salivary proteins. As discussed earlier, evidence of declined levels of calcium in healthy elderly along with the reduced salivary protein secretion thus may significantly affect oral health in elderly population (Al-Hashimi and Levine, 1989; Lendenmann et al., 2000; Nagler and Hershkovich, 2005). Therefore, the lack of mucins may not only lead to oral disease due to lack of defence but also dryness of the oral mucosa.

3.3 MATERIAL PROPERTIES OF SALIVA

Saliva, as a material, is generally characterized by measuring its viscosity, elasticity, adherence and spinnbarkeit (Gohara et al., 2004; Stokes and Davies, 2007). The first step in the study of saliva as a research material is the collection of saliva and its preservation. Saliva properties differ as a function of the collection method (Stokes and Davies, 2007; Proctor, 2016). Many studies demonstrated distinct results (e.g. rheology, tribology) when using stimulated versus unstimulated saliva (Prinz et al., 2007; Silletti et al., 2008). In addition, the difference of salivary stimulation is magnified in the elderly. For example, mild stimulation, such as use of lemon-drop resulted in 39% age-dependent decrease in salivary flow rate (Pedersen et al., 1985). While, using strong stimuli, such as pilocarpine hydrochloride, the capability of salivary glands in drug-free elderly was also reduced (Nagler and Nagler, 1999; Nagler and Hershkovich, 2005). Once the saliva was collected, changes in the composition naturally occur due to the proteolysis and bacterial metabolism (Gohara et al., 2004; Schipper et al., 2007; Takehara et al., 2013; Wagner and McKinley, 2017). In order to minimise the contamination, pre-treatments (e.g. centrifugation, dehydration) and storage conditions (e.g. freezing) are required in saliva preservation. However, these actions also lead to changes in some salivary parameters. For example, with the storage time and after being frozen, the salivary viscosity was found to decrease significantly (Stokes and Davies, 2007).

The macromolecules i.e. mucins affect the rheological properties of saliva. Shear viscosity (i.e. energy dissipated during flow) using simple capillary viscometer (Waterman et al., 1988) and elasticity (i.e. energy stored) using uni-axial elongational flow (Zussman et al., 2007) have been measured in saliva from elderly individuals. An age-related reduction in salivary flow rate, accompanied by an increase in salivary viscoelasticity and protein content were reported (Figure 4). This age-dependent increase in salivary viscosity was also further supported using elongational thread viscometer by Kazakov et al. (2009).

[Figure 4 here]

Currently oral tribology is emerging as a promising tool to quantify friction and lubrication of food-saliva mixtures in the oral mucosa using in vitro polymeric set up (Laguna et al., 2017a; Laguna and Sarkar, 2017; Laguna et al., 2017b). Studies involving Xerostomia patients have reported reduced sulfation of salivary mucins (both MUC1 and MUC2), which may affect the lubricating property of saliva (Chaudhury et al., 2016). However, there is no tribological work published to date, regarding the change of saliva with age. Considering that mucin, calcium content and mono-valent ionic concentration of saliva differs significantly in elderly individuals as compared to young population, lubrication and adsorption properties of saliva are likely to be affected in elderly individual. This might affect the textural perception of food significantly, however, such hypothesis needs to be validated with well-coordinated instrumental and sensory studies with healthy elderly subjects.

The loss of negatively-charged glycan residues transforms mucins from extended polymers into more tightly packed globular aggregates, which causes a reduction of water retention capacity of mucin in the residual saliva and oral dryness (Coles et al., 2010). This may also provide an explanation for patients suffering from dry mouth conditions even with high mucin concentrations (Saari et al., 1997). Furthermore, the degradation of sulfation of mucins is proposed as the causative factors in changes of rheological properties of saliva (Yamada, 1980) (Figure 4). On the other hand, the spinnability (fibrosity) of saliva measures the adhesive and elastic properties of saliva to enable its adsorption to the oral surfaces and form a pellicle (Vijay et al., 2015). Studies have indicated that unstimulated saliva in dry mouth sufferers has significantly lower spinnability. This is largely attributed to the reduction as well as degradation of moisture-retaining mucin proteins (Carpenter, 2013; Chaudhury et al., 2015). On the other hand, the highly concentrated and viscous saliva lacks the ability to flow freely in the oral cavity (Zussman, Yarin, & Nagler, 2007). This results in the localized areas of dryness and followed by the perception of dry mouth (Närhi, 1994). These age-dependent changes in quality and quantity of saliva is summarized systematically in Figure 4. This suggests that the treatments for dry mouth patients and rational design for artificial saliva should include not only the quantity, but also the ability of saliva to be retained on surfaces.

4. EFFECT OF SALIVARY CHANGES ON FOOD FLAVOUR PERCEPTION IN OLDER ADULTS

All non-volatile food components need to be dissolved in the saliva in order to reach the taste receptors (Neyraud, 2014). Furthermore, the role of saliva is also implicit in mouth sensations caused by food components, such as astringency that occurs by the complexation of food polyphenols and salivary proteins (Brossard et al., 2016; Rutuja et al., 2016; Laguna and Sarkar, 2017). On the other hand, the effect of concentration changes of inorganic ions might elevate the taste thresholds and decrease supra-threshold intensities that give an explanation of elderly people suffering from taste aberrations. It has been also shown that this age-degenerated taste sensitivity is not only related to the decrease of number of taste buds (Shin et al., 2012), but also of the salivary cell production resulting in a time lag in the turnover of taste receptor cells. It is well-recognized that the response to sucrose and sweetness perception is pH-dependent. Hence, changes in salivary inorganic composition resulting in the changes of salivary pH can explain its influence on the receptor's stimulation indirectly affecting sweetness perception in elderly population (Tierney and Atema, 1988; Matsuo and Yamamoto, 1992).

Although it has not been linked with old age, changes in the salivary flow has shown a significant positive correlation between saliva flow and time to reach maximum intensity of sweetness and cherry flavour in chewing gums (Guinard et al., 1997). Therefore, age-dependent changes in salivary composition can have a direct influence on taste perception and might consequently affect food intake (Boesveldt et al., 2018). In a systematic review (Muñoz-González et al., 2018), it was inferred that salivary hypofunction was associated with a decrease of the objective chewing and swallowing abilities and taste perception. Interestingly, rare attention has been paid in literature to investigate the relationship between salivary flow and texture or aroma on one side and salivary composition, taste/texture perception and food intake on the other side. This suggests that more research is needed in this field to understand the role of hyposalivation on potential decline of aroma/ texture perception and its consequences on food intake.

5. CONCLUSIONS AND FUTURE REMARKS

It is clear that age-related changes in saliva are multifactorial. On one hand, a reduced salivary flow rate has an influence on increasing the ionic concentration of saliva, inadequate levels of fluid to coat the oral cavity and reduced ability to cope with the rate of pellicle replenishment. On the other hand, the decrease of salivary mucins and calcium might render loss of the lubricating properties of bio-lubricant saliva, which can result in reduced wettability of oral surfaces eventually leading to xerostomia, associated oral symptoms and potential aberrations in sensorial and/or textural perception. There is still a need to study the changes of salivary properties with age, especially regarding its role as a lubricant. For instance, changes (if any) in coefficient of friction, apparent viscosity and viscoelasticity of saliva in healthy elderly population due to the decrease in mucin and other organic components, calcium, increase in other ions in elderly subjects still remains to be elucidated. Furthermore, how such salivary changes affect sensorial/ textural perception and consequently food intake remain to be quantified, which is critical for food design for the elderly population. And considering salivary properties changes significantly upon freezing, thawing and accurate standardized protocols are required for such experiments to ensure adequate repeatability.

It is worth noting that other age-related factors are equally important to account for the salivary changes. With ageing, the prevalence of age-related disease, such as Alzheimer, cancer and some chronic diseases increase which have been indicated to result in salivary hypofunction directly (Ship et al., 1990; Sadeghi et al., 1996; Nagler et al., 1997; Ship et al., 2002). Also, due to these diseases, elderly population require an increased number of medications and clinical treatments (Gao et al., 2018). It was reported that, more than 87% elderly (age over 65 y.o.) has taken at least one medication regularly (Rochon, 2016). Many of the drug therapies would usually affect saliva through anticholinergic activity (Mandel, 2008; Rochon, 2016). Life-style factors, such as smoking habits would also modify saliva gradually over time (Rad et al., 2010). However, the complicated relationships between ageing in healthy individuals, age-related health conditions and salivary changes still remain open for discussion. Many researchers indicate the importance of health conditions to account for the variability of results observed in salivary studies conducted in elderly subjects (Nagler and Hershkovich, 2005; Nassar et al., 2014; Affoo et al., 2015; Vandenberghe‐Descamps et al., 2016).

In summary, the key limitations identified in researches conducted in saliva in ageing population are as follows:

1. Lack of details of salivary collection. It is well-recognized that different salivary collection protocols, such as stimulation, preservation, individual variations, health condition of the individuals can create significant variation in data. Majority of studies in this area lack precise details of salivary collection, which is crucial for validation purposes.
2. Limited number of subjects. Larger samples of participants need to be pooled in salivary researches in elderly population to increase the statistical power of significance (Österberg et al., 1992).
3. No distinct age range. Considered the objective of studies regarding age-dependent changes in saliva, the priori and commonly accepted definitions of younger and older participants are especially important. The variety of the age ranges among different studies can impact the results significantly. Furthermore, some studies have insufficient age-subdivision when investigating influence of

age on saliva changes, for example, 10-24 years old, 25-39 years old, 40-54 years old, 55-69 years old have been used in previous reports (Österberg et al., 1992; Challacombe et al., 1995; Salvolini et al., 2000).

4. Data analysis. The methods and criteria used for analysing data can provide huge deviation to results. For example, in the study by Affoo et al. (2015), removal of outlier resulted in a 11% decrease in the effect size for unstimulated salivary flow rate. Therefore standardised study design and statistical comparison methods should be considered.
5. Factors other than age-related processes in elderly population. Saliva secretion and age-dependency is a complex multi-factorial problem. For example, many researchers found no significant difference between elderly group whom regularly take medication compared with drug-free elderly group regarding salivary flow rate and salivary composition, although both show significant differences with respect to younger group (Nagler and Hershkovich, 2005). To date, literature is scarce on relationship between saliva, age, polypharmacy and oral sensorial complaints and/or textural and sensorial aberrations.

Future research should be conducted addressing all these issues and use standardize protocols for saliva collection. Research should be also dedicated to understand relationship between salivary quality/ quantity, textural/ aroma sensation and food intake in the elderly population. With carefully planned and suitable control procedures, such as use of age groups, healthy versus elderlies with health conditions and/or type of medication used, the knowledge base on age-dependent change in quantity and quality of saliva will be expanded offering potential application to designing optimized food and oral therapies for maintaining optimal oral health and nutritional status in the elderly population.

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Captions of Figures

Figure 1. Human salivary glands. Adapted from Varga (2015).

Figure 2. Histological samples from parotid glands of a) young individual (23 y.o.) and b) old individual (83 y.o.). In a) the acini are even and compactly arranged without evidence of degenerative changes. In b) the acini are uneven in size, shape and staining and there is more interstitial fibrous tissue. Reproduced from Scott *et al.* (1987).

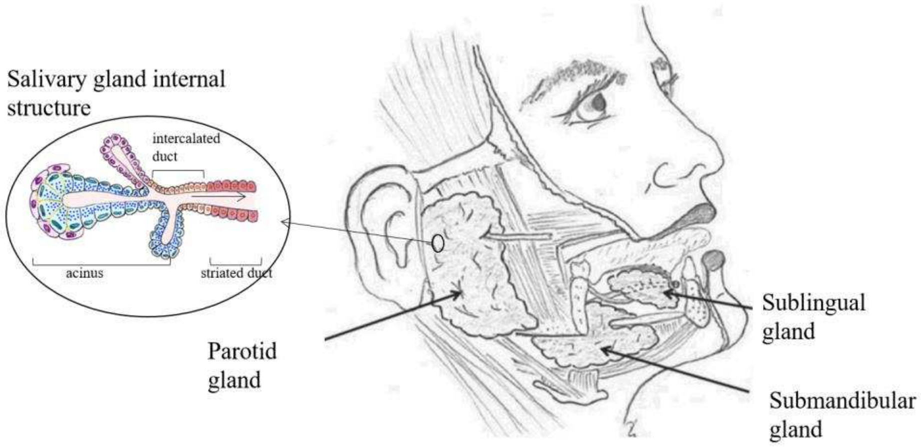
Figure 3. SDS-PAGE of unstimulated whole mouth saliva samples, following centrifugation from (a) patients with dry mouth and (b) healthy controls. Samples stained using (i) Coomassie Brilliant Blue R250, (ii) Periodic acid–Schiff polysaccharide staining for detecting glycocomponents of mucin glycoproteins following SDS-PAGE, (iii) MUC5B and MUC7 mucin protein detection by Western blotting, and (iv) Sialic acid residues being detected using 2 lectins, M is the marker. Reproduced from (Chaudhury *et al.*, 2015).

Figure 4. Summary of age-related changes in quantity and quality of saliva.

Table 1. Significant changes* in human unstimulated whole salivary composition reported in elderly population with respect young population. Elderly populations > 60 years old in all studies.

		Reported significant increase ↑ or decrease ↓ in concentration	References
Saliva components	Inorganic	↑ Potassium (K^+) ↑ Chloride (Cl^-) ↑ Phosphate (P) ↓ Calcium (Ca^{2+}) ↑ Uric acid	(Nagler and Hershkovich, 2005; Nassar et al., 2014)
	Organic	↑ **Lysozyme** ↑ Amylase ↑ IgA ↓ MUC1, MUC2 ↓ Lactoferrin, Transferrin ↓ Reduced and oxidized glutathion ↓ Peroxidase activity	(Chang et al., 2011; Denny et al., 1991; Nagler, 2004; Nagler and Hershkovich, 2005; Navazesh et al., 1992; Salvolini et al., 2000)

*Note: Statistical significance is represented by $p\text{-value} < 0.05$.



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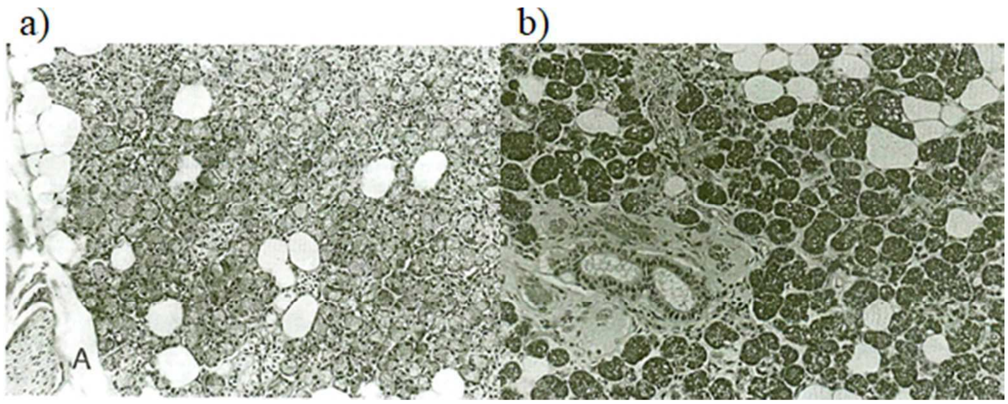

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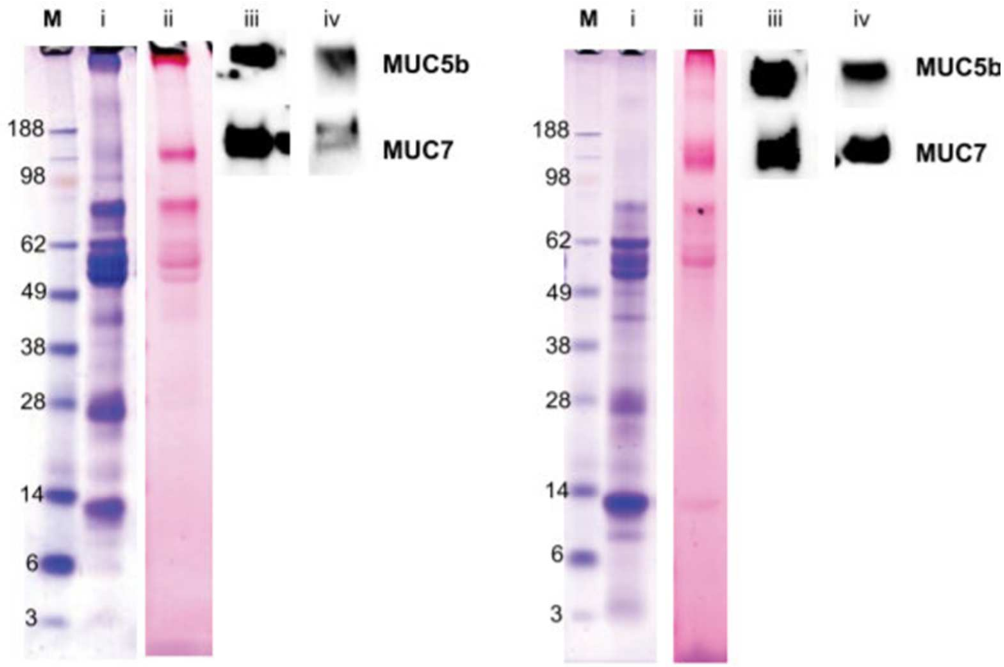

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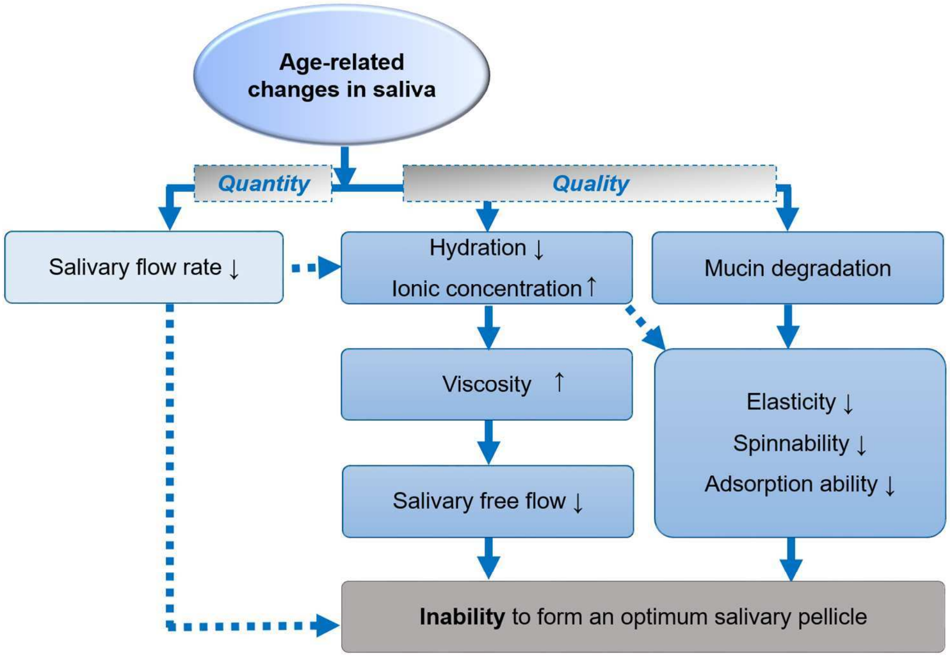

Figure 4. Summary of age-related changes in quantity and quality of saliva.